# GENETIC ANALYSIS OF SELECTED SEED CONSTITUENT TRAITS IN CHICKPEA (*Cicer arietinum* L.)

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# ABSTRACT

Seed composition is a major factor that influences grain utilization and end-use. To improve chickpea (*Cicer arietinum* L.) seed quality, it is imperative to identify novel genetic resources with desired seed composition for use in breeding programs. The specific objectives of the study were: 1) to characterize natural variation for selected chickpea seed composition traits; and 2) to identify the chickpea genomic regions associated with variations in seed constituent traits. The study is based on the hypothesis that natural variation exists for chickpea seed composition and it is associated with specific genomic regions. Seed composition characters such as one thousand seed weight (TSW), protein, starch and amylose concentrations were analyzed in three distinct chickpea germplasm collections grown in multiple environments. The study utilized three distinct germplasm collections: (i) a reference (237 genotypes); (ii) composite (168 genotypes) and (iii) a recombinant inbred lines (RIL, 224 genotypes) grown in multiple environments. All the three chickpea germplasm collections showed variability in seed composition traits. The multiple environment testing also revealed strong effects of genotype by environment interaction on the selected quality traits showing a high broad sense heritability for TSW (0.65 – 0.87) and medium to low heritability for total starch (0.13 – 0.48), protein (0.16 – 0.57) and amylose (0.11 – 0.17). The negative correlation of TSW and total starch with amylose and protein complicates the direct selection for a trait of interest. Therefore, a compromise needs to be made to select genotypes that exhibit a relatively balanced seed composition. Three desi (ICC 16903, ICC 4958 and ICC 93954), two kabuli (ICC 7255 and ICC 8261), and one pea-shaped accession (ICC 8350) were identified that showed desired seed composition and consistent performance across the environments. The composite collection was genotyped by the Diversity Array Technology (DArT) and the RIL population was genotyped by genotyping by sequencing (GBS) to identify genomic regions associated with seed composition traits. The association mapping study with the composite germplasm collection using 380 DArT markers identified two sub-populations that were also confirmed by Principal Coordinate Analysis (PCoA). The mixed linear model identified 33 marker-trait associations for all the traits in both desi and kabuli accessions, explaining 4.2 – 10.3 % variance for TSW, 3.7 – 16.1 % for total starch, 5.1 – 9.0 % for protein, and 4.1 – 11.0 % for amylose, respectively. The bi-parental RIL mapping population analyzed by GBS identified 415 single nucleotide polymorphisms (SNP) that identified eight linkage groups. Six quantitative trait loci (QTLs) for TSW explained 2.5 – 24.6 % of total variance, four QTLs

explained 2.5 – 19.4 % of phenotypic variance for protein and one QTL for total starch and amylose explained 18.6 % and 8.3 % of phenotypic variance, respectively. QTL robustness was low for amylose. Epistatic effects were low and did not affect the common QTLs. Within the identified QTLs, seven putative genes were associated with the phenotypic variation observed in the RILs. These 33 marker-trait associations (MTAs) and putative genes need to be further studied to develop molecular markers that can be utilized in marker assisted selection (MAS) to accelerate the development of chickpea genotypes with desired seed composition. The results support the hypothesis that chickpea germplasm varies for chickpea seed composition and it is associated with specific regions of chickpea genome.

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| | | |
|---|---|---|
| AFLP | : | Amplified Fragment Length Polymorphism |
| AMOVA | : | Analysis of Molecular Variance |
| ANOVA | : | Analysis of Variance |
| BC | : | Backcross |
| cM | : | Centi-Morgan |
| CIM | : | Composite Interval Mapping |
| CV | : | Coefficient of Variation |
| DArT | : | Diversity Array Technology |
| DH | : | Doubled Haploid |
| F | : | Field Trial |
| $F_2$ | : | Second Filial Generation |
| FAO | : | Food and Agriculture Organization |
| G × E | : | Genotype by Environment Interaction |
| GBS | : | Genotyping by Sequencing |
| GH | : | Greenhouse Trial |
| GLM | : | Generalized Linear Model |
| GOPOD | : | Glucose Oxidase/Peroxidase |
| GWAS | : | Genome Wide Association Study |
| $H^2$ | : | Broad Sense Heritability |
| ICARDA | : | International Center for Agricultural Research in the Dry Areas |
| ICIM | : | Inclusive Composite Interval Mapping |
| ICRISAT | : | International Crop Research Institute of Semi-Arid Tropics |
| IQR | : | Inter-Quartile Range |
| LD | : | Linkage Disequilibrium |
| LG | : | Linkage Group |
| LOD | : | Log of Odd |
| MAGIC | : | Multi-parent Advanced Generation Intercross |
| MAS | : | Marker Assisted Selection |
| MET | : | Multiple Environment Trial |
| Mbp | : | Mega Base Pairs |
| MIM | : | Multiple Interval Mapping |

| | | |
|---|---|---|
| MLM | : | Mixed Linear Model |
| MTA | : | Marker Trait Association |
| MUFA | : | Mono-Unsaturated Fatty Acid |
| NGS | : | Next Generation Sequencing |
| NIL | : | Near Isogenic Line |
| $P$ | : | Probability |
| PCoA | : | Principal Co-ordinate Analysis |
| PCR | : | Polymerase Chain Reaction |
| PIC | : | Polymorphism Information Content |
| PVE | | Phenotypic Variation Explained |
| PUFA | : | Poly-Unsaturated Fatty Acid |
| QEI | : | QTL by Environment Interaction |
| QTL | : | Quantitative Trait Locus |
| QTN | | Quantitative Trait Nucleotide |
| RAPD | : | Random Amplified Polymorphic DNA |
| RFLP | : | Restriction Fragment Length Polymorphism |
| RIL | : | Recombinant Inbred Line |
| SMA | : | Single Marker Analysis |
| SDI | : | Shannon-Weaver Diversity Index |
| SIM | : | Simple Interval Mapping |
| SNP | : | Single Nucleotide Polymorphism |
| SSR | : | Simple Sequence Repeat |
| TSW | : | Thousand-Seed Weight |
| UFA | : | Unsaturated Fatty Acid |
| $\sigma_e^2$ | : | Error Variance |
| $\sigma_g^2$ | : | Genotypic Variance |
| $\sigma_{gl}^2$ | : | Genotype by Environment Interaction Variance |
| $\sigma_p^2$ | : | Phenotypic Variance |

# CHAPTER 1. INTRODUCTION

## 1.1 Background

Pulse crops are grain legumes, whose dry seeds are harvested and marketed distinguishing them from vegetable legumes. The term "pulse" was derived from the Latin word "*puls*" or "*pultis*" meaning "thick soup" or "potage". Seven major pulse crops contribute to 89 % of global pulse production. Another thirteen pulses are less commonly grown and contribute to 11 % of global pulse production (Chibbar et al., 2010). Globally, among the major pulse crops, chickpea (*Cicer arietinum* L.) accounted for 18.3 % of the total production quantity and 17.3 % of the total seeded land in more than 50 countries, making it the second most important pulse crop after dry beans (FAOSTAT, 2014). The cultivated chickpeas are of two distinct types: Desi and Kabuli. Both types of chickpea have been acclimated to the western Canada's brown to dark brown soil zones (Pulse Canada, 2015). In western Canada chickpea was first cultivated in 1993 (Pulse Canada, 2015). In 2014 crop year, Canada contributed 0.12 million tonnes to the global chickpea production (FAOSTAT, 2014). Saskatchewan and Alberta are the two main chickpea producing provinces in Canada, with Saskatchewan accounting for more than 80 % of the Canada's chickpea production (Pulse Canada, 2015).

Globally chickpea is a highly sought after pulse due to its nutritional value and as an inexpensive source of protein. Chickpea seed contains 51 to 71 % of carbohydrates, 13 to 30 % of protein, 6 to 9 % of lipids and minor quantities of vitamins, minerals, phytochemicals and low concentrations of anti-nutritional substances (Muzquiz and Wood, 2007; Wood and Grusak, 2007). Compared to cereals, chickpea protein is deficient in methionine but rich in lysine, and therefore, chickpea makes an ideal diet complement to cereals. Most (85 %) of the chickpea lipids are unsaturated fatty acids (UFA) with poly-unsaturated fatty acids (PUFA) constituting 66 % and mono-unsaturated fatty acids (MUFA) 19 %. In addition to the major seed storage constituents, chickpea is also a good source of vitamins B and E, and minerals, such as Fe (5.0 mg/100 g), Zn (4.1 mg/100 g), Mg (138 mg/100 g) and Ca (160 mg/100 g) (Jukanti et al., 2012). Anti-nutrients can be reduced by heating and other seed processing methods (Gaur et al., 2007). As the major component of storage carbohydrates, starch accounts for 30 to 60 % of chickpea's dry seed weight. Chickpea starch is inherently rich in amylose ranging from 30 to 40 % of the total starch (Chibbar et al., 2010; Jukanti et al., 2012).

Chickpea seed quality depends on both external and internal factors. The external factors include grain appearance, whereas the internal factors are determined by the seed

storage constituents. Chickpea seed storage compounds, including protein, starch and amylose, and minor components are significantly influenced by genotype, environment and their interaction (Rubio et al., 1998; Sirohi et al., 2001; Frimpong et al., 2009; Alwawi et al., 2010; Dehghani et al., 2010). There are very limited reports in the literature related to chickpea seed quality improvement (Gaur et al., 2007).

Global chickpea breeding programs that are focused on agronomic and disease resistance traits, have seen major advancements over the past few years (Gaur et al., 2007). Chickpea lines of high or medium Ascochyta blight resistance (Bakhsh et al., 2005; Basandrai et al., 2007; Sreelatha et al., 2008; Ghazanfar et al., 2010; Sharma et al., 2010; Pande et al., 2011; Pande et al., 2013), *Fusarium oxysporum* resistance (Sharma et al., 2012), *Botrytis cinerea* resistance (Sharma et al., 2013a), pod borer resistance (Sreelatha et al., 2008), and tolerance to abiotic stresses (Krishnamurthy et al., 2011) have been developed. With advances in molecular biology and genomics, molecular markers have been identified and utilized to assist in plant breeding for rapid selection of useful breeding genetic resources and to accelerate the development of improved cultivars (Gaur et al., 2012; Gaur et al., 2014). Genetic maps have been well established with a variety of molecular markers (Gaur et al., 2014). QTLs/genes have been discovered for resistance/tolerance against biotic (tolerance to Fusarium wilt, Ascochyta blight, Botrytis grey mold), and abiotic stresses (salinity and drought tolerance), and traits of agronomic importance (days to flowering, days to maturity, seed coat thickness, seed size and pod number) in chickpea (Sharma et al., 2004; Radhika et al., 2007; Anbessa et al., 2009; Gowda et al., 2009; Anuradha et al., 2011; Rehman et al., 2011; Vadez et al., 2012). However, literature regarding the improvement of chickpea seed nutritional quality or the QTLs/genes analysis of phenotypic variation for seed nutritional composition are very limited. Therefore, studies on the association of seed nutritional composition and genomic variation are needed. These studies will facilitate the understanding of genetic control of chickpea seed quality and the development of novel breeding strategies for chickpea seed quality improvement.

### 1.2 Hypothesis

Further to the previous research on nutritional quality improvement of chickpea seed, the present study is based on the following hypotheses:

1. Natural variation exists in chickpea seed for traits such as TSW, seed protein, total starch and amylose.
2. The selected seed constituent traits are associated with chickpea genomic regions.

## 1.3 Objectives

The objective of this project is to characterize and understand the genetic basis of natural variation in selected chickpea seed constituents. The main objective will be achieved as the following three sub-objectives:

1. To characterize natural variation for thousand seed weight (TSW) and selected seed constituent traits such as total starch, amylose and protein concentrations in chickpea.
2. To discover marker-trait association for the selected seed constituent traits.
3. To identify genomic regions associated with the selected seed constituent traits.

# CHAPTER 2. LITERATURE REVIEW

## 2.1 Chickpea

### 2.1.1 Taxonomy

Chickpea (*Cicer arietinum* L.) is an annual diploid with 16 chromosomes and genome of 740 Mbp (Jain et al., 2013). Chickpea is a member of genus *Cicer*, tribe *Cicereae*, subfamily *Papilionoideae*, family *fabaceae* (Maesen et al., 2007). The genus *Cicer* is divided into two subgenera (*Pseudononis* Popov and *Viciastrum* Popov), four sections (*Monocicer* Popov, *Chamaecicer* Popov, *Polycicer* Popov and *Acanthocicer* Popov) and 14 series according to different botanical characteristics (Maesen, 1972). The genus *Cicer* includes 44 recognized species around the world, however, only one species (*C. arietinum* L.) is cultivated extensively in 58 countries (Maesen et al., 2007; FAOSTAT, 2014).

### 2.1.2 Classification

Based on distinct botanical or morphological features and molecular diversity analysis, chickpea is primarily classified into two major groups: desi type and kabuli type (Moreno and Cubero, 1978; Iruela et al., 2002). Desi (microsperma) type chickpea is characterized by the small size seeds, pods, leaflets and plantlets. Nevertheless, large variations in flower and seed coat color, and seed shape are commonly observed. The flower color ranges from white to red, purple and blue, and seed coat with different shades of brown. The seed shape is angular, and seed coat varies from smooth to wrinkled which reflects the genetic diversity (Cobos et al., 2007; Redden and Berger, 2007) (Figure 2.1 A and C). The “macrosperma” chickpea, the kabuli type, is known for the “ram’s head” shaped seeds. In contrast to the desi type, the kabuli type is characterized by large seeds, pods, leaflets and plantlets. Flower and seed coat colors are usually white or cream colored. The seed coat of kabuli type is thinner and less winkled than that of the desi type (Redden and Berger, 2007; Wood et al., 2011) (Figure 2.1 B and D). A rare chickpea type, known as “pea shaped” (Figure 2.1 E), constitutes about 3.8 % of ICRISAT’s chickpea germplasm collection. They have intermediate morphologies between desi and kabuli types: small seeds and pods, smooth seed coat, round seed shape like pea seed, with a range of seed coat color (Upadhyaya, 2003).

### 2.1.3 Origin and domestication

Chickpea is one of the “founder crops” that participated in the evolution of agriculture


A B

C D E

**Figure 2.1** Chickpea plants and seeds.
**A**, desi chickpea plant with flower; **B**, kabuli chickpea plant with flower; **C**, desi chickpea seeds; **D**, kabuli chickpea seeds; **E**, the pea-shaped chickpea seeds.

in the "fertile crescent" (Abbo et al., 2010; Zohary et al., 2012). Chickpea domestication started around 8000 – 5450 BC in the adjoining regions of southeast Turkey and northeast Syria (Redden and Berger, 2007). Evidence of chickpea remains during excavations at the prehistoric sites, revealed that besides some undistinguishable chickpea species, most were *C. arietinum* from the early Neolithic age, 9300 BC to the recent 200 AD (Redden and Berger, 2007).

Analyses of seed proteins, isozymes and karyotype suggest that the contemporary domestication of chickpea occurred through the hybridization of *C. arietinum* with a wild species *C. reticulatum* (Ladizinsky and Adler, 1976). Chickpea domestication caused major changes in the morphology (reduction in pod dehiscence, larger seed and plantlet size), anthocyanin pigmentation, and physiology by reducing/eliminating dormancy and vernalization that resulted in change from winter to summer habit (Ladizinsky and Adler, 1976; Smartt, 1984; Abbo et al., 2003). The summer habit of domesticated chickpea caused temporal reproductive isolation from other wild species of winter habit, thus contributing to the reduced genetic diversity of chickpea (Abbo et al., 2003).

### 2.1.4 Geographical distribution

By the early Neolithic Age, chickpea distribution had been restricted to the "fertile crescent" (Bar-Yosef, 1998). In the following Bronze Age, chickpea was distributed to Crete in the west, north Egypt in the south, and through Iraq to the Indian subcontinent in the east. By the Iron Age, the domesticated chickpea had spread all over the south and the west Asia. At the same time, the first record of chickpea uses appeared in Ethiopia (Redden and Berger, 2007). Portuguese and Spanish travelers brought the domesticated chickpea back to Europe in 1500 AD (Maesen, 1972). The kabuli type was introduced from the southwestern Europe and northwestern Africa to the Indian peninsula around 1700 AD (Maesen, 1972). The desi type might have been introduced to the eastern Africa by the immigrants from the Indian subcontinent in the last decades of 1800 AD (Maesen, 1972). At present chickpea is cultivated in 58 countries around the world forming different centers of distribution (Figure 2.2).

### 2.1.5 Chickpea production in the world and Canada

The worldwide production of chickpea in 2012 was 13.12 million tonnes with a seeded area of 13.57 million ha, second to that of dry bean that ranked first among the pulse crops (22.81 million tonnes and 29.05 million ha) (FAOSTAT, 2014). The top ten chickpea producing countries include India, Australia, Pakistan, Turkey, Myanmar, Iran, Ethiopia, Mexico, Canada, and the USA, producing 8.83, 0.81, 0.75, 0.51, 0.49, 0.30, 0.25, 0.21, 0.17, and 0.16 million

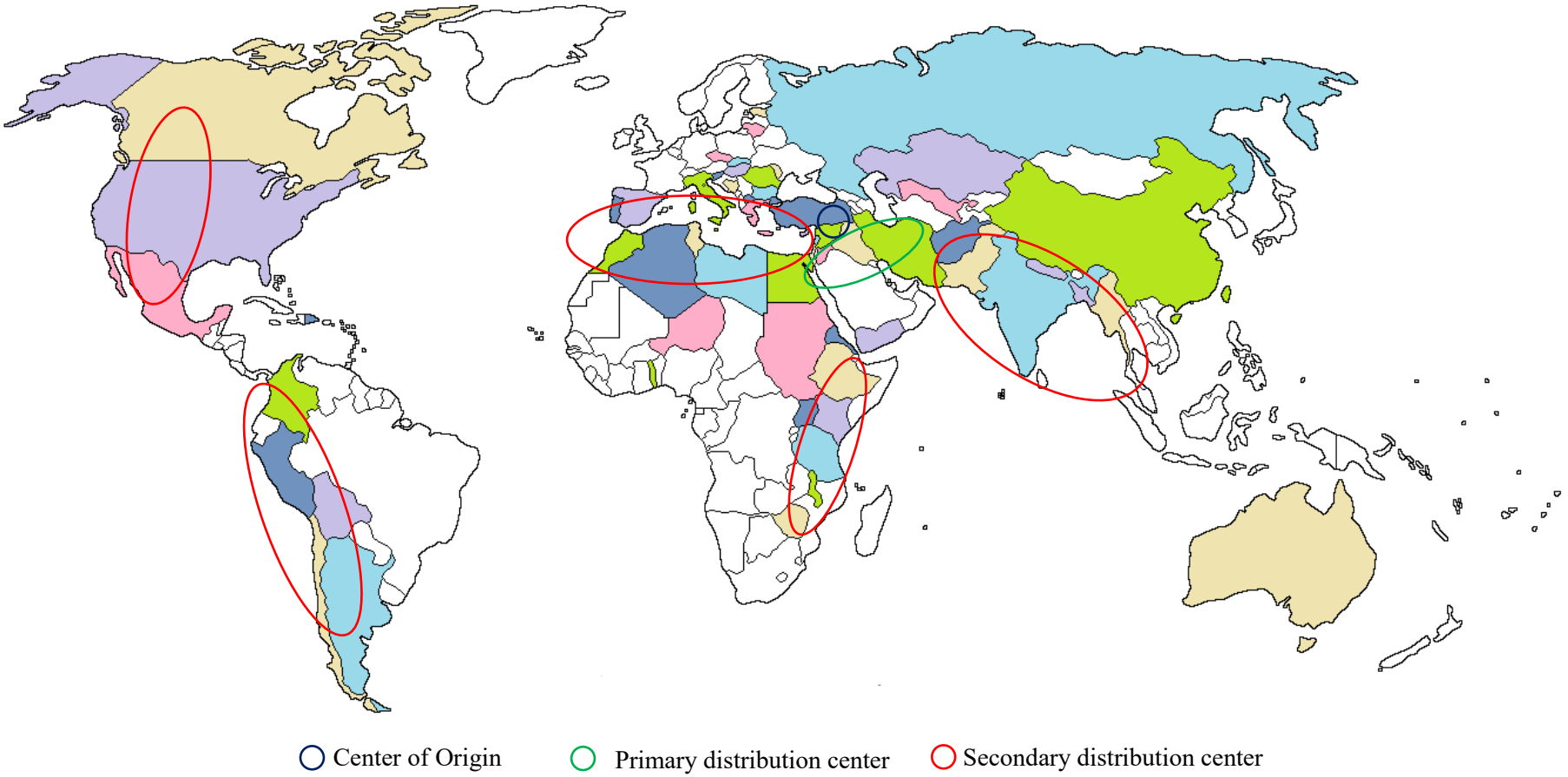


**Figure 2.2** World map to show the chickpea center of origin, distribution centers and major chickpea producing countries (colored regions).
Source: FAOSTAT (2014).

tonnes, respectively. Almost 70 % of the global chickpea production is contributed by India, while Canada contributes 2 % (FAOSTAT, 2014). As a major pulse crop producer in Canada, Saskatchewan produced 0.12 million tonnes of the chickpeas in 2013, and in 2014 (Pulse Canada, 2015).

## 2.2 Chickpea germplasm

### 2.2.1 Germplasm collection

Genetically diverse germplasm is a source of useful traits in chickpea improvement programs. The International Crop Research Institute for the Semi-Arid Tropics (ICRISAT, Hyderabad, India) houses 20,602 chickpea accessions collected from 62 countries worldwide, including India (7,677), Iran (5,295), Turkey (973), Ethiopia (960), Afghanistan (734), Pakistan (723), Mexico (457), Syria (447), Nepal (349), Morocco (304) and other regions (ICRISAT, 2015). At ICARDA (International Center for Agriculture Research in the Dry Areas, Syria), there are 12,070 chickpea genotypes mainly collected from Iran (1,780), Turkey (970), India (410), Chile (340), Uzbekistan (300), Spain (280), Tunisia (270), Morocco (230), Bulgaria (210), Portugal (170) and other regions. Other chickpea gene banks include USDA (United States Department of Agriculture) and ATFCC (Australian Temperate Field Crop Collection) that possess 900 and 670 chickpea genotypes, respectively (Redden and Berger, 2007).

### 2.2.2 Core collection

The germplasm collection normally contains all the available genetic diversity of chickpea from around the world. However, the large population size and the high level of individual commonalities render it inefficient to screen for phenotypic diversity for use in a breeding project. Therefore, to efficiently screen the natural genetic variation, Frankel (1984) proposed the concept of “core collection” to minimize repetitiveness of the accessions in a germplasm collection. A core collection includes hierarchical groups according to taxonomy and geographic regions. At ICRISAT, a chickpea core collection that is 11 % of their total germplasm includes 1,956 accessions, composed of 1,465 Desi, 433 Kabuli and 58 Intermediate types based on their origin and 13 quantitative characteristics (Upadhyaya et al., 2001).

### 2.2.3 Mini-core collection

To further reduce the number of accessions in a core collection and to preserve variation of individuals, a second stage selection is applied to develop a ‘mini-core’ collection.

Upadhyaya and Ortiz (2001) assessed the 1,956 genotypes of the chickpea core collection for biological and agronomical characteristics and seed quality to develop a mini core collection of 211 genotypes that was approximately 11 % of the core collection. Analysis for means, variances, and frequency distribution of several characters in the mini core collection reflected the diversity in the core collection.

### 2.2.4 Global composite collection and reference set

In a collaborative project between ICRISAT and ICARDA, 3,000 chickpea accessions were evaluated to develop a global composite collection (Upadhyaya et al., 2006). The objective for such a global composite collection was to identify genotypes carrying specific alleles conferring chickpea beneficial traits. A reference set is a subset of the global composite collection, and is composed of ~300 genotypes (10 % of the composite collection) which exhibit the highest genetic diversity (Upadhyaya et al., 2008).

## 2.3 Nutritional composition of chickpea seed

Compared with other legume crops, chickpea has a relatively balanced nutritional composition (Jukanti et al., 2012). It is a major food source for people living in the semi-arid regions. It provides protein for vegetarians and people who are unable to afford animal protein in the region. Chickpea also supports the daily need of carbohydrates and other nutrients (Table 2.1). Chickpea carbohydrates comprise up to 60 % of dry seed mass. Protein ranges from 20 to 29 %. Lipids are a minor constituent in major seed storage compounds, accounting for 4 to 6 %. Fibers range from 4 to 20 % depending on different plant materials. Whereas, minerals comprise up to 3.5 %.

### 2.3.1 Carbohydrates

Carbohydrates have a basic empirical formula $C_x(H_2O)_y$, but most of the carbohydrates in plants are present as oligomers or polymers of simple and modified sugars. Based on the degree of polymerization of simple sugars, carbohydrates are grouped into monosaccharide, disaccharide, oligosaccharide, and polysaccharide (Chibbar et al., 2016). Based on the ease of digestibility and absorption in the human gut, dietary carbohydrates are divided into two classes: available and unavailable carbohydrates. The available carbohydrates are digested and easily absorbed in the small intestine, while the unavailable carbohydrates are not digested in the small intestine but fermented by the microflora in the colon. The available carbohydrates include mono- and di- saccharide, starch, while the unavailable carbohydrates consist of

**Table 2.1** Major seed constituents in chickpea.

| Seed constituents | Khan et al. (1995) | | Rincon et al. (1998) | | Viveros et al. (2001) | | Maheri-Sis et al. (2008) | | Sharma et al. (2013b) | |
|---|---|---|---|---|---|---|---|---|---|---|
| (%) | Desi | Kabuli | Desi | Kabuli | Desi | Kabuli | Desi | Kabuli | Desi | Kabuli |
| Carbohydrate | 47.5 | 55.0 | 47.4 | 47.6 | 51.0 | 49.0 | 46.8 | 49.1 | 60.7 | 52.7 |
| Protein | 25.4 | 24.4 | 21.5 | 21.7 | 25.0 | 29.0 | 22.8 | 24.6 | 21.4 | 29.0 |
| Lipid | 3.7 | 5.1 | 3.1 | 4.6 | 4.5 | 6.0 | - | - | 4.5 | 5.0 |
| Fiber | 11.2 | 3.9 | 21.6 | 19.5 | 9.0 | 6.0 | 9.9 | 6.5 | 4.9 | 3.8 |
| Ash | 3.2 | 2.8 | 3.2 | 3.4 | 3.2 | 3.0 | - | - | 3.5 | 3.5 |

oligosaccharides, resistant starch, pectins, hemicelluloses, cellulose and non-cellulosic polysaccharides (Chibbar et al., 2010; Jukanti et al., 2012). The indigestible carbohydrates are fermented by microflora in the large intestine producing short chain fatty acids, and releasing carbon dioxide, methane and other gases. Carbohydrates are considered as the major storage component in chickpea, varying from 51 to 65 % in desi type and from 54 to 71 % in kabuli type (Wood and Grusak, 2007).

### 2.3.2 Starch

Starch is the major storage carbohydrate in pulse grains (Chibbar et al., 2010). Chickpea seed contains 30 – 57 % starch which serves as the main dietary energy source (Wood and Grusak, 2007). Starch is constituted by two distinct types of glucose polymers: amylose ($10^5$ – $10^6$ Da) and amylopectin ($10^7$ – $10^8$ Da) differing in properties and stored as water insoluble discrete granules (Chibbar et al., 2010). Amylose is a linear molecule of 100 – 10,000 glucosyl units interconnected by α-(1,4) linkages with sparse branches, which constitutes 20 – 41 % and 23 – 47 % of a starch granule in desi and kabuli, whereas amylopectin is a large, highly and regularly branched glucan polymer resulting from the α-(1,4) and α-(1,6) linkages between the linear glucan chains and side chains, respectively (Chibbar et al., 2010; Jukanti et al., 2012) (Figure 2.3 A and B). Compared with amylose, amylopectin has a relatively complex side chain structure and several models have been proposed in the past decades (Tetlow, 2010). However, cluster model (Bertoft, 1986) is most commonly accepted. The side chains of amylopectin exhibit different polymorphic forms in the lamellae essential to the structure of starch granule (Jukanti et al., 2012) (Figure 2.3 C – E). Chickpea seed contains 29.0 – 54.7 % of total starch with 26.9 – 39.0 % of amylose depending on different genotypes and amylose determination methods (García-Alonso et al., 1998; Singh et al., 2004; Kaur and Singh, 2006; Frimpong et al., 2009; Hughes et al., 2009).

### 2.3.3 Protein

Chickpea seed protein contents range between 12.6 and 30.5 %, which is around two – fold higher than cereal grains. Wood and Grusak (2007) reported that protein content of chickpea seed ranged from 16.7 g/100 g to 30.6 g/100 g, and 12.6 g/100 g to 29.0 g/100 g for desi and kabuli, respectively. In other studies, kabuli had higher protein content than desi and some found no significant difference in protein content (Jukanti et al., 2012). This could be due to genotype or differences in cultural practices.

In terms of amino acid composition, limited concentration of sulphur amino acids and

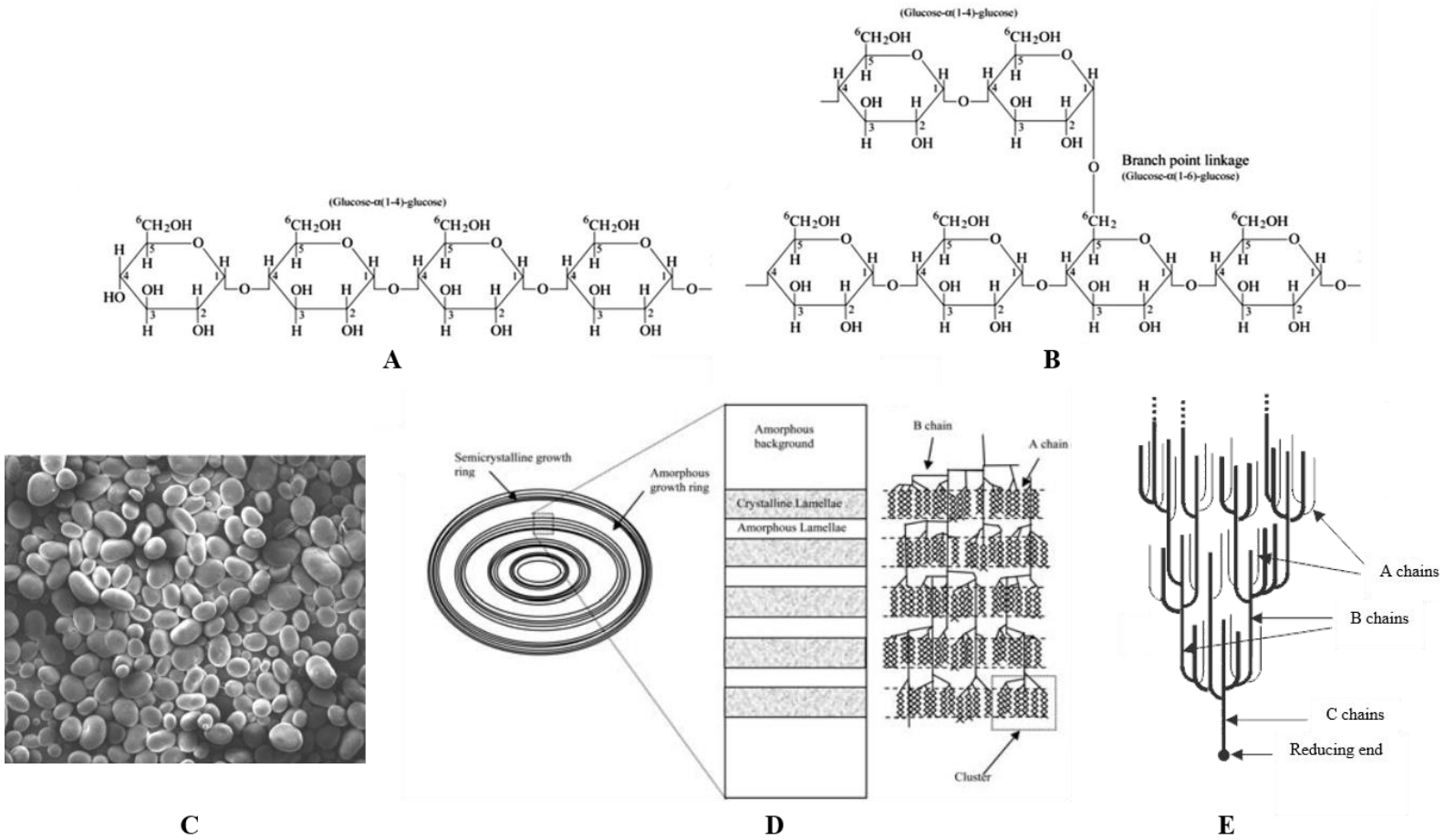


**Figure 2.3** Representation of molecular structures of starch and starch granule.
An amylose molecule has a long chain of glucose residues connected through the α(1-4) linkages with very few side chains branched by α(1-6) linkages (**A**). Whereas, an amylopectin molecule differs with a large number of side chains and a short main chain (**B**). Amylose and amylopectin are stored in starch granules (**C**, chickpea starch granules in scanning electron micrograph). Starch granules are made up of semi-crystalline growth rings andd amorphous growth rings; the semi-crystalline rings contain alternating crystalline lamellae and amorphous lamellae; A chains of an amylopectin molecule forming clusters, constitute the crystalline lamella whereas the region between two adjacent A chain clusters make the amorphorous lamella where amylose molecules are present (**D**). A chains form double helix strusture with hydrogen bonds through the interaction of adjacent chains; B-chains connect all A-chains to C-chain through α(1-6) glucosidic linkages; and C-chain is a short chain and the only one chain that has the reducing end on an amylopectin molicule (**E**). Source: Buleon et al. (1998), Miao et al. (2009), Hamley (2010), Fasahat et al. (2014), and Chibbar et al. (2016).

abundance in lysine make chickpea an ideal companion to cereal based products in human diet (Chibbar et al., 2010). Amino acid profile in chickpea seed shows that there are nine types of essential amino acids. Leucine and lysine are the highest constituents, ranging from 6.3 – 7.8 g/100 g and 5.6 – 8.8 g/100 g, and 5.5 – 8.9 g/100 g and 6.7 – 7.6 g/100 g in desi and kabuli, respectively. Whereas, methionine and tryptophan are the lowest in concentration. Desi has 0.9 – 2.3 g/100 g of methionine and 0.6 – 1.1 g/100 g of tryptophan, while in kabuli methionine ranges from 1.3 g/100 g to 2.5 g/100 g and tryptophan ranges from 0.6 g/100 g to 0.8 g/100 g. Chickpea has a good amount of non-essential amino acids except cysteine that ranges from 0.2 g/100 g to 2.8 g/100 g and from 0.2 g/100 g to 2.9 g/100 g in desi and kabuli, respectively (Zia-Ul-Haq et al., 2007; Liu et al., 2008; Wang et al., 2010b; Malunga et al., 2014).

Chickpea seeds contain higher concentrations of essential (39.89 g/100 g protein) and endogenous amino acids (58.64 g/100 g protein) compared to wheat (32.20 and 56.55 g/100 g protein, respectively). Supplementation of sorghum flour to chickpea flour showed increased concentrations of essential amino acids, lysine, methionine, cysteine and tyrosine in the mixture (Rachwa-Rosiak et al., 2015).

### 2.3.4 Lipids

The total lipid content of chickpea ranges from 4.5 to 6.0%. Chickpea features high levels of essential unsaturated fatty acids, linoleic acid (54.7 – 56.2 %), oleic acid (21.6 – 22.2 %), and linolenic acid (0.5 – 0.9 %), whereas low levels of saturated fatty acids, palmitic acid (18.9 – 20.4 %) and stearic acid (1.3 – 1.7 %). The total lipid contents of desi and kabuli types range from 2.9 to 7.4 % and 3.4 to 8.8 %, respectively (Jukanti et al., 2012).

### 2.3.5 Minor nutrients

Chickpea is considered as a good source of minerals, such as Ca, P, Mg, Fe, and K. In 100 g of dry seed mass, there are 165.0 mg of Ca, 994.5 mg of K, 169.0 mg of Mg, 4.59 mg of Fe, 451.5 mg of P, 4.07 mg of Zn, and 3.81 mg of Mn for desi type; however, kabuli type has some discrepancies in mineral content compared to desi type (Wang et al., 2010a).

Vitamins are another group of minor nutrients which are essentially involved in metabolism. Chickpea seeds contain both the water soluble vitamins B and C, and the lipid soluble vitamins A, E and K. Chickpea has abundant amounts of vitamin $B_3$ (niacin) and pyridoxine (a form of vitamin $B_6$) (Wood and Grusak, 2007).

### 2.3.6 Health benefits

Chickpea has a 12,000 year history of utilization as a human food. However, during the past two or three decades, the impact of chickpea nutrients on human health has been recognized (Jukanti et al., 2012). Recent reports on chickpea nutritional benefits are associated to multiple vitamins, minerals, and other bioactive compounds. However, chickpea carbohydrates especially the higher concentration of amylose in starch and dietary fiber has been suggested to reduce the incidences of obesity and diseases such as type 2 diabetes, cardiovascular diseases and colorectal cancer, while promoting digestive health (Chibbar et al., 2010; Jukanti et al., 2012).

## 2.4 Determination of starch properties

### 2.4.1 Seed starch extraction

An accurate and reproducible method of starch extraction from grains is needed to determine its physicochemical characters (Zhong et al., 2009). Complete removal of residual proteins, lipids and other small molecules from starch is a major challenge in starch extraction methods. Co-purified compounds can interfere with determination of starch and amylose concentrations and their physicochemical properties.

A common starch extraction method for wheat was described by Zhao and Sharp (1996) and modified by Demeke et al. (1999). Wheat seeds were steeped overnight in distilled water at 4 °C. Water was decanted and the softened seeds were ground into slurry. The slurry was transferred onto cesium chloride (80 % w/v) and centrifuged at 13000 ×g for 5 min. This step was repeated twice to remove lipids and proteins. Starch granules were washed twice with Tris-HCl buffer (pH 6.8) and air-dried overnight.

Unlike cereal grain starch, pulse starch isolation is difficult due to the presence of insoluble flocculent protein and fibers. The substances always sediment with starch exhibiting a brownish deposit on top of the extracted starch (Hoover and Sosulski, 1991). To isolate chickpea and horse bean starch, the pulse flour was steeped overnight (Lineback and Ke, 1975). Residual pulp was re-washed and rescreened with distilled water and a 60-mesh sieve. Starch was centrifuged at 2000 ×g for 20 min, supernatant decanted, brown upper layer removed with a spatula and starch was air-dried at room temperature. Miao et al. (2009) steeped chickpea grains in 0.2% sodium hydrogen sulphite overnight at 20 °C. The steeped grains were ground into slurry that was filtered with a 100-mesh sieve and centrifuged at 3000 ×g for 20 min. The sediment was washed completely with distilled water and the process was repeated until starch was free of color. Finally, the starch was oven-dried at 40 °C for 12 hours.

### 2.4.2 Total starch determination methods

The most widely acceptable starch determination method is the AACC approved method (AACC Method 76.13). In this method, starch is sequentially degraded by thermostable α-amylase followed by amyloglucosidase. The released glucose is quantified with glucose oxidase/peroxidase reagent (GOPOD) by measuring absorbance at 510 nm using a spectrophotometer (McCleary et al., 1994).

### 2.4.3 Amylose determination methods

Amylose content in starch influences the physicochemical characteristics including retrogradation, gelation rate, gelation temperature, and solubility. Therefore, determination of amylose concentration is important. The iodometric method was the first method to determine amylose concentration (Mahmooda et al., 2007). Iodine is able to form complex with both amylose and amylopectin, however the complexes with different molecules give different colors. The blue color indicates amylose complexed with iodine whereas the reddish brown reflects the binding of iodine to amylopectin. The blue and the reddish brown colors have maximum absorptions at 620 nm and 540 nm, respectively. Based on the color intensity, the amylose content is determined. This method can be used for rapid measurement in a large number of samples. However, the results from this method are usually complicated by the interaction of amylose and fatty acids, and long chains of amylopectin.

A lectin, concavalin A interacts with non-reducing groups of amylopectin and amylose. Amylopectin having large numbers of non-reducing end-groups, can be precipitated with concavalin A apart from amylose (Gibson et al., 1997). The amylopectin precipitation can be removed through centrifugation and amylose remaining in the supernatant can be determined by measuring the free glucose released after hydrolysis. However, pH, ionic strength and temperature needs to be optimized for complete precipitation of amylopectin which is very complicated, time-consuming and major drawback of the method (Demeke et al., 1999).

Amylose can be precisely determined by a high performance size exclusion chromatography (HP-SEC) (Demeke et al., 1999). In this method, amylopectin is debranched with isoamylase and the debranched starch mixture is separated by a size exclusion based high performance liquid chromatography linked to a refractive index detector. The relative concentrations of amylose and amylopectin can be calculated as peak areas. Although this method is very informative but it is very time-consuming and takes at least an hour for each sample.

## 2.5 Genotype by environment interaction and chickpea seed composition

### 2.5.1 Effect of genotype by environment interaction on different traits in chickpea

Several studies have reported the effect of genotypes (G), environments (E), and their interaction on different agronomic traits in chickpea (Appendix 1) including grain yield and its components, and disease resistance. Most studies showed very strong effects of G × E interaction, whereas a few did not show a significant influence of G × E interaction due to limited numbers of environments and genotypes. There are very few studies establishing the effect of G × E interaction on seed constituent traits (Appendix 2). Singh et al. (1983) and Frimpong et al. (2009) reported G×E effects on major seed storage compounds, but only few genotypes or environments were included. Therefore, an extended study with large number of genotypes and environments needs to be performed.

### 2.5.2 Heritability

Heritability ($H^2$) of a trait in a population of a species can be affected by the interaction of genotype and environment. By definition, heritability represents the phenotypic variation resulting from genotypic variation:

$H^2 = V_G \,/\, V_P$ ……(2.1),

$V_G$ stands for the total genotypic variation resulting from additive gene effect, dominance and epistasis. $V_p$ represents the total phenotypic variation consisting of total genotypic variation and environmental variation. Additionally, the environmental variation includes the general environmental variation, G×E interaction variation, and specific environmental variation.

Broad sense heritability ($H^2$) and narrow sense heritability ($h^2$) are distinguished by the variation caused by different gene effects. The broad sense heritability emphasizes the total genotypic variation, whereas the narrow sense heritability only focuses on the variation caused by the additive gene action.

Broad sense heritability ($H^2$) can be calculated from an ANOVA table as described by Singh et al. (1993):

$H^2 = \sigma_G^2 \div (\sigma_G^2 + \sigma_I^2 + \sigma_e^2)$ ……..(2.2)

$\sigma_G^2 = (M_G - M_I) \div (b \times L)$ ……...…(2.3)

$\sigma_I^2 = (M_I - M_e) \div b$ ……(2.4)

$\sigma_e^2 = M_e$ ……(2.5)

Thus, the $H^2$ can be calculated as,

$H^2 = (M_G - M_I) \div [M_G + M_I \times (L-1) + M_e \times (b-1) \times L]$ .....(2.6)

In the formula, $M_G$, $M_I$ and $M_e$ stand for the mean squares (MS) from the ANOVA table for genotype, G×E interaction and error, whereas b is the number of block (replication), and L represents the number of environment. The broad sense heritability ($H^2$) ranges from 0 to 1. $H^2$ values < 0.3, 0.3 – 0.6 and > 0.6 are considered as low, medium and high heritabilities, respectively (Gangola et al., 2013). Higher $H^2$ represents the consistent performance of the trait across multiple environments.

## 2.6 Genetic mapping

Advances in DNA technologies combined with molecular markers revealing DNA polymorphisms, enabled the transition of genetics from observable phenotypes to the underlying genetic regulation. The progress can assist in the efficient selection of elite genotypes to accelerate the development of new varieties with desired phenotype.

Genetic mapping was introduced in the late 1980s to understand the genetic basis of quantitative traits (Lander and Botstein, 1989). Major advancements in molecular biology techniques led to rapid and cost-effective genotyping that is useful to identify genomic regions affecting quantitative traits in most crops of interest (Ingvarsson and Street, 2011). Genetic mapping mainly deals with the identification and localization of genomic regions (Quantitaive Trait Locus or QTL) associated with a trait of interest, and the estimation of phenotypic variance explained by the the identified QTL in specific populations.

At present, linkage mapping and association mapping are the two most commonly used genetic mapping strategies to dissect genetic bases of complex traits. Both approaches attempt to locate genomic regions underpinning trait variation by using statistical means to discover co-inheritance of genes or loci with genetic markers (Oraguzie and Wilcox, 2007).

### 2.6.1 Linkage mapping

#### 2.6.1.1 Mapping populations

To develop a linkage mapping population, two parents with contrasting phenotypes are needed. The selection of two suitable genotypes of a species is the first step towards the successful development of a mapping population. The parents are considered to be appropriate when they are genetically divergent, produce fertile progenies, show adequate polymorphisms in genetic markers, and exhibit a medium level of segregation distortion (Semagn et al., 2006b). A mapping population can be doubled haploids (DHs), second filial generation ($F_2$), backcross (BC), near isogenic lines (NILs) and recombinant inbred lines (RILs) for self-pollinating species (He et al., 2001; Doerge, 2002). Population type and size can influence the accuracy of

linkage maps. Insufficient numbers of individuals of a mapping population results in fragmented linkage groups and aberrant gene orders. Co-dominant markers produce more accurate linkage maps for RILs and $F_2$ than for other types of population. Whereas, maps with dominant markers are better for RILs, BC, DHs and NILs than for $F_2$ mapping population. A high resolution genetic map requires a large number of individuals and polymorphic markers (Ferreira et al., 2006).

#### 2.6.1.2 Linkage mapping methods

Several linkage mapping methods have been reported to identify QTLs such as single marker analysis, simple interval mapping and multiple QTL mapping (Shaukeen, 2015).

The single marker analysis (SMA) is also called marker regression analysis, and it is the simplest method that allows rapid scan of QTLs (Soller et al., 1976). In this approach, offspring are grouped into various classes according to marker genotypes. The average of the marker genotype groups is estimated and compared using T-test. The marker may be linked to a QTL if significant difference is detected in phenotypic effect between marker genotype classes (Soller et al., 1976). SMA is limited because markers linked to one QTL may be linked to another QTL and this cannot be determined using this approach. QTLs distant from markers cannot be detected and QTL effects are underestimated. Thus, its accuracy is lower than that of other methods (Jansen and Stam, 1994).

Simple interval mapping (SIM) is a better method than SMA for QTL analysis. SIM uses markers flanking the putative QTLs to estimate their locations, thus, increasing the probability of identifying the QTLs. SIM uses likelihood ratio test to determine every QTL position in the interval created by flanking markers (Lander and Botstein, 1989). SIM is considered a better method than SMA because the evidence for a QTL is visualized with a curve, the position of the QTL is determined by support intervals, the estimation of QTL effect is improved, and data with missing marker genotype information can also be analyzed (Broman, 2001). Nevertheless, SIM can deal with only one QTL at a time on a linkage group, and therefore it becomes less effective when several QTLs are contained in the same linkage group (Lander and Botstein, 1989; Broman, 2001)

To overcome the multiple QTL problem with SIM, Jansen (1993) and Zeng (1993) proposed that regression analysis should be introduced to SIM. Zeng (1993) named it as composite interval mapping (CIM). In CIM, markers linked to other QTLs are taken into consideration, enhancing the power to detect multiple QTLs by reducing error variance. When QTL mapping models are extended to a multiple QTL model, they become even more powerful

and accurate to identify QTLs. This method is called Multiple QTL mapping (MIM). MIM takes into consideration the effects of multiple QTLs on the one under investigation and simultaneously incorporate several putative QTLs into the model (Kao et al., 1999). MIM may have avoided the complicated background selection problem with CIM but the advantage still exists. For example, MIM introduces different background selection models, however, with different models, different mapping results are generated (Li et al., 2007). Inclusive composite interval mapping (ICIM) was proposed by Li et al. (2007) to improve the conventional CIM by performing background marker selection only once with stepwise regression of all marker information simultaneously. ICIM simplified the computation, increased QTL detection power and minimized false positives or biased QTL effects (Li et al., 2007). Most notably, ICIM can analyze epistatic interaction between QTLs and QTL by environment interaction (QEI) (Li et al., 2008).

### 2.6.2 Association mapping

Linkage mapping has been established as an extremely useful technique to identify genomic regions underlying varying phenotypic expressions of quantitative traits (Ingvarsson and Street, 2011). However, it has some limitations. To overcome the limitations of linkage mapping, association or linkage disequilibrium (LD) mapping has been developed to dissect complex traits in plants (Barhen et al., 1995). LD is the non-random associations between loci. Association study identifies the non-random associations between markers and adaptive traits in natural populations (Nordborg and Weigel, 2008). Population structure in the natural population, is a very strong confounding factor in an association mapping study (Nordborg and Weigel, 2008).

Assocaiton mapping has several advantages over linkage mapping. Linkage mapping uses highly pedigreed family lines arising from controlled crosses with two genetically distinct parental lines (Semagn et al., 2006b). Creating such populations can take five to ten years to reach the appropriate generation for linkage analysis. However, association mapping uses a natural population with a wide genetic background. The natural population usually have more than two families with a small family size (Wurschum, 2012). The diverse genetic backgrounds of natural population enable the simultaneous analysis of several alleles and traits compared to the linkage analysis where structured populations are studied for only one or a few pairs of alleles per locus at a time if they are present in the parental lines (Wurschum, 2012).

Both the mapping methods utilize the power of recombination events. However, QTLs in linkage mapping, due to limited number of crossing overs during the population

development, are usually localized in 10 – 30 cM intervals of flanking markers that may contain 20-30 million base pairs coding for hundreds of genes resulting in low mapping resolution (Oraguzie et al., 2007; Zhu et al., 2008). Association mapping has higher mapping resolution compared to linkage mapping as natural populations are derived from common ancestors, and therefore, have undergone a large number of historical and revolutionary recombination events (Zhu et al., 2008). Therefore, instead of detecting gene loci where a quantitative trait nucleotide (QTN) is present, association study can identify causative sequences as close as possible to the QTN (Oraguzie and Wilcox, 2007). Therefore, a very high number of markers are required for an association mapping study (Oraguzie and Wilcox, 2007).

The use of bi-parental populations in linkage mapping to identify QTL restricts its use in marker assisted selection (MAS) schemes due to the specificity of the QTL in the population used in the study (Holland, 2007; Bernardo, 2008). In association mapping, the detected QTL effects have wider applicability as these have been developed from population of genetically diverse individuals. In addition, other differences between linkage and association mapping include that markers and QTLs co-segregate as expected in Mendelian laws in bi-parental mapping population. However, in natural populations of association mapping, markers could segregate from nearby markers at different frequencies. Association study can also search whole genome for specific sequences and/or candidate genes for polymorphisms (Oraguzie and Wilcox, 2007).

### 2.7 Molecular marker systems

Molecular markers are detectable polymorphic DNA sequences inherited to the next generation (Semagn et al., 2006a). Based on the analysis method, molecular markers can be divided into two groups: hybridization-based and PCR-based molecular markers (random amplified polymorphic DNA, RAPD; amplified fragment length polymorphism, AFLP; inter-simple sequence repeat, ISSR; microsatellite; expressed sequence tags, EST; cleaved amplified polymorphic sequence, CAPS; sequence characterized amplified region, SCAR; sequence tagged site, STS; single nucleotide polymorphism, SNP; diversity array technology, DArT) (Semagn et al., 2006a).

The PCR-based genotyping methods can be classified into two categories: (a) the semi-/arbitrarily prime PCR polymerization without known genomic information, and (b) the site-targeted PCR techniques with prior DNA sequence information. The former includes RAPD, AFLP, and ISSR. The latter consists of EST, CAPS, SCAR, and STS. RFLP, RAPD, ISSR, AFLP and SSR have been utilized widely for genetic research in plants (Semagn et al., 2006a).

In this study, the two molecular marker systems that were used are discussed below.

### 2.7.1 Diversity array technology (DArT)

Diversity Array Technology (DArT) is a microarray hybridization-based method that simultaneously analyzes several hundred polymorphic loci distributed over a genome (Jaccoud et al., 2001; Reinke and Kilian, 2004; Xie et al., 2006). DArT marker analysis requires no prior DNA sequence information, and as a high-throughput genotyping technique DArT is time-saving, highly reproducible and cost-effective. However, DArT cannot distinguish heterozygotes and needs complicated platform demanding extensive investment in laboratory facilities (Semagn et al., 2006a).

### 2.7.2 Genotyping by sequencing to identify single nucleotide polymorphism (SNP)

Single Nucleotide Polymorphism (SNP) is the variation for a single nucleotide in DNA sequence among individuals of a species. In terms of marker abundancy, SNP has the largest number across a genome compared to other molecular marker systems. SNP markers can be used to determine genetic diversity efficiently, especially for speiceis with low level of genetic diversity. The binary nature of a SNP marker enables it to competently differentiate homozygotes from heterozygotes (Arif et al., 2010). The power of SNP markers arises from the large number of accessible loci, whereas the power of simple sequence repeat (SSR) markers is derived from the number of alleles (Foster et al., 2010). SNP markers are evolutionarily conserved which avoids some of the influences of homoplasy (Brumfield et al., 2003). Moreover, SNPs are amenable to high throughput automation, enabling rapid and efficient genotyping of large sample numbers (Tsuchihashi and Dracopoli, 2002). Therefore, they are considered as the next genereation marker system.

Progresses in next-generation sequencing (NGS) simplified genotyping by sequencing (GBS) of plants with large and highly diverse genomes (Elshire et al., 2011). GBS can detect large numbers of SNPs through genotyping and computational genetic analysis (Beissinger et al., 2013). Cost effective and easy sample preparation protocols, no reference genome limitation, thrifty barcoding and easy scale-up are the major advantages of GBS (Davey et al., 2011). Consequently, GBS is being utilized in population/plant/animal genetics, diversity studies and breeding programs (Poland et al., 2012).

## 2.8 Genetic mapping in chickpea

### 2.8.1 Mapping populations

Genetic mapping studies in chickpea has been performed mostly using RILs and sometimes with $F_2$ or other mapping populations (Gaur and Slinkard, 1990a, b; Kottapalli et al., 2009). Recently, a multi-parent advanced generation intercross (MAGIC) population was developed at ICRISAT (Gaur et al., 2014). The eight parents of this MAGIC population are cultivars and superior breeding lines collected from India and Africa. The eight parents underwent 28 two-way, 14 four-way and seven eight-way crosses to create the final population (Gaur et al., 2012). The multi-parent mapping population brings large numbers of recombination events to the progenies resulting in higher genetic diversity and higher mapping resolution (Glaszmann et al., 2010). The MAGIC population derived from large numbers of recombination events would become a novel source of plant material for chickpea improvement (Gaur et al., 2012).

### 2.8.2 Molecular markers and QTLs in chickpea

The very first genetic map of chickpea was constructed by Gaur and Slinkard Gaur and Slinkard (1990b). At that time, the genetic map was developed with 26 isozyme markers for two traits in chickpea morphology and the map consisted of seven linkage groups. Subsequently, DNA-based molecular markers were introduced to construct genetic maps for chickpea (Simon and Muehlbauer, 1997). At present, linkage maps of chickpea have been well established with various DNA-sequence-based molecular markers (Appendix 3). Among the genetic studies, QTL analyses for biotic/abiotic resistance are the most dominant. Genes/QTLs identification for agronomic traits such as seed yield, seed/pod number and growth habit have also been done. However, QTL studies for seed constituent traits in chickpea are limited. Therefore, studies need to be carried out on the seed composition to improve chickpea seed nutritional quality and enhance its economic value.

## 2.9 Seed quality improvement for chickpea

Chickpea provides an excellent source of starch and proteins. Chickpea, having adequate amounts of sulphur-rich amino acids, makes a nutritional supplement to cereals and other starchy crops, especially in vegetarian diets. Although chickpea oil content cannot compete with other oilseeds, the polyunsaturated fatty acids account for up to 65 % of the total oil concentration. Minerals such as calcium, phosphorus, zinc, iron and magnesium are also more abundant in chickpea. Additionally, chickpea is also an inexpensive source of vitamins,

especially the high levels of vitamins B, E, and carotenoids. Therefore, as a good source of protein and energy as well as a cheap source of micronutrients, chickpea is gaining popularity as a healthy food among people from both the developing and developed countries.

There have been extensive studies on breeding for biotic and abiotic resistant chickpea lines, but limited efforts have been made to improve the nutritional quality of chickpea seed. Therefore, to develop breeding strategies to improve chickpea nutritional quality, the diversity in seed composition among chickpea germplasm needs to be evaluated. In addition, genomic regions associated with chickpea seed composition can result in molecular markers that may be used in marker assisted breeding to accelerate the development of chickpea cultivars with enhanced nutritional benefits.

# CHAPTER 3. GENOTYPE, ENVIRONMENT AND THEIR INTERACTION INFLUENCE SEED CONSTITUENT TRAITS IN CHICKPEA (*Cicer arietinum* L.)

## 3.1 Study 1*

This study used a chickpea reference set to determine natural variation in chickpea thousand seed weight, protein, starch and amylose concentration. This study also analyzed genotype × environment interaction of the selected seed composition characteristics.

* Wang, R., Gangola, M. P., Jaiswal, S., Gaur, P. M., Båga, M., and Chibbar, R. N. 2016. Genotype, environment and their interaction influence seed constituent traits in chickpea (*Cicer arietinum* L.) (Submitted to *Journal of Food Composition and Analysis*).

## 3.2 Introduction

Chickpea (*Cicer arietinum* L.) is an annual diploid plant (2n = 2x = 16), and the only one cultivated species of the genus *Cicer* (Family Fabaceae) (Maesen et al., 2007). During 2014, after dry bean chickpea was the second most important pulse crop in the world with a total production of 14.2 million tonnes cultivated over 14.8 million hectares of seeded area in more than 50 countries (FAOSTAT, 2014). Chickpea has two major commercial classes: desi (purple flower, wrinkled thick seed coat, and small, dark, angular seeds) and kabuli (white flower, smooth thin seed coat and large, cream-colored seeds) types that are usually grown in semiarid tropical and temperate regions of the world, respectively (Gangola et al., 2013). A third intermediate type chickpea is termed as "pea-shaped" and characterized by small to medium seed size, and cream to brown colored round seeds (Upadhyaya et al., 2008).

Chickpea has gained popularity and importance especially in developing countries, and is also being accepted by the developed world (Jukanti et al., 2012). The popularity of chickpea in human diet is mainly attributed to relatively low price and balanced seed nutrients composition. In the semi-arid regions, chickpea seed serves as the common protein source for vegetarian people and those who cannot afford animal protein (Muehlbauer and Rajesh, 2008). Starch and protein are the two major compounds of chickpea seeds, constituting about 80 % of the total dry seed mass (Jukanti et al., 2012). Starch concentration ranges from 30 to 60 % of the total dry seed mass in chickpea (Wood and Grusak, 2007). Amylose constitutes about 30 – 40% of total starch content, and helps to prevent diabetes, heart disease and obesity (Chibbar et al., 2010; Jukanti et al., 2012). Chickpea seeds also contain higher concentrations of protein (12.6 – 30.6 %) compared to cereals (5.8 – 15.0 %) (Wood and Grusak, 2007). Chickpea seed protein has a well-balanced amino acid composition; however, sulphur-rich amino acids (methionine and cysteine) are limited but lysine is abundant compared to cereals that are rich in methionine and cysteine but limited in lysine. Consequently, chickpea and cereals are a good diet companion (Leterme and Munoz, 2002). Lipid concentration varies from 2.9 to 8.8 % in chickpea seeds, about 66 % of which is poly-unsaturated fatty acids (Jukanti et al., 2012). These suggest the nutritional importance of chickpea seeds.

Seed composition is controlled genetically (G), but it is also influenced by growing environment (E) and its interaction with the genotype (G × E) (Hood-Niefer et al., 2012; Gangola et al., 2013). In chickpea, G × E affects both agronomic (Malhotra and Singh, 1991; Bakhsh et al., 2006; Imtiaz et al., 2013) and seed constituent traits (Frimpong et al., 2009; Alwawi et al., 2010). Chickpea seed constituents, such as starch and protein, play an important role in chickpea utilization, processing and end-user acceptance.

To improve chickpea seed quality and aid in developing effective breeding strategies, plant materials should show variation for traits of interest in natural environments. The present study tests that natural variation occurs in the selected seed constituent traits. Therefore, natural variation was evaluated in a collection of diverse chickpea genotypes for important seed constituent traits as influenced by G, E and G × E. The study also identified chickpea genotypes with good seed constituent traits and stable multi-environment performance to provide genetic resources for breeding programs for seed quality improvement.

## 3.3 Material and methods

### 3.3.1 Plant material

To study natural variation for selected seed constituent traits, a collection of 237 chickpea genotypes (180 desi, 49 kabuli and 8 pea shaped) was procured from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, Patancheru, India). These genotypes were collected from 26 countries covering four continents throughout the world (Appendix 4).

Chickpea genotypes were grown in three trials with two replications each: two field trials in 2011 (F 2011) at ICRISAT (17 ° 53 ′ N latitude, 78 ° 27 ′ E longitude and 545 m altitude, Patancheru, India), in 2013 (F 2013) at Aberdeen (52 ° 34 ′ N latitude, 106 ° 29 ′ W longitude and 517 m altitude, SK, Canada) whereas one greenhouse (GH) trial in 2012 (G 2012) at University of Saskatchewan (52 ° 07 ′ N latitude, 106 ° 38 ′ W longitude and 481.5 m altitude, Saskatoon, SK, Canada). Field experiments were performed in a randomized complete bock design during October to mid-March and June to mid-September at ICRISAT and Aberdeen, respectively. Seeds of each lines were sown with 10 and 30 cm spacing between plants and rows, respectively. Fertilizers (22 kg/ha $P_2O_5$ and 40 kg/ha N) were applied before seeding. For F 2011, mean minimum and maximum temperatures were 15.5 and 31.7 °C, respectively, with 7.2 mm of average precipitation during the growing season. The mean minimum and maximum temperatures were 11.1 and 23.0 °C, respectively, for F 2013 with a mean precipitation of 2.3 mm. In the GH, average daily minimum and maximum temperatures were 18 and 23 °C with an 18 h photoperiod. Plants were harvested and seeds were stored at room temperature. All genotypes were grown in two blocks. In each block, 50 seeds of one genotype were sown in a single row. When harvested, all seeds from the 50 plants of the same genotype were bulked. For each genotype, the selected seed constituent traits were analyzed two times for each block, giving two biological replicates for each block. For each biological replicate in each block, two technical replicates were analyzed, giving eight technical replicates in total. Field trial of 2011

was carried out by researchers and employees at ICRISAT and seeds were sent to the University of Saskatchewan for analysis.

### 3.3.2 Seed weight analysis

One hundred seeds of each genotype were counted using electronic seed counter (Seedburo Equipment Co., Chicago, IL, USA) and weighed. The weight was multiplied by ten to obtain one thousand seed weight (TSW).

### 3.3.3 Grinding of seed material

Chickpea seeds (about 10 g) were ground into a fine meal using a UDY cyclone mill (Udy Corporation, Fort Collins, CO, USA) to pass through a 0.5 mm sieve. The seed meal was collected, stored at room temperature and used to determine total starch, amylose and protein concentration.

### 3.3.4 Determination of total starch concentration

Total starch concentration in chickpea seed meal (100 ± 0.5 mg) was determined by an enzymatic hydrolysis method using a commercial kit (Megazyme International Ireland Ltd., Wicklow, Ireland) (McCleary et al., 1997). Starch was sequentially hydrolyzed into dextrins and finally to D-glucose using α-amylase and amyloglucosidase, respectively. The D-glucose was treated with glucose oxidase/peroxidase reagent (GOPOD) producing red quinoneimine, the concentration of which was determined at A510 nm.

### 3.3.5 Amylose determination

Starch was purified, prior to amylose determination, from chickpea seed meal following a modified method (Peng et al., 1999) using cesium chloride (CsCl) density gradient centrifugation. Chickpea seed meal (200 ± 1 mg) was suspended in distilled water (5 mL), vortexed, filtered into a 15 mL disposable tube. The disposable tube was centrifuged at 3,000 × *g* for 10 min and supernatant was discarded. The sediment was placed on the top of cesium chloride (1 mL of 80 % v/v solution) in a 2 mL centrifuge tube. The tube was centrifuged at 16,000 ×*g* for 30 min. Supernatant was discarded; and the pellet was washed sequentially with distilled water, wash buffer [contained 55 mL of Tris-HCl (1 M; pH 6.8), sodium dodecyl sulfate (23 g), glycerol (100 mL) and distilled water (final volume up to 1 L)] and distilled water (twice) at 16,000 × *g* for 10 min. The pellet was finally washed with acetone (1 mL), centrifuged at 16,000 × *g* for 10 min and air-dried at room temperature.

The purified starch was used to determine amylose concentration using an iodine based method with some modifications (Mahmooda et al., 2007); therefore, it is expressed as percentage of total starch. In brief, purified starch (5 mg) was weighed in a 2 mL centrifuge tube. The starch was sequentially suspended in 95% (v/v) ethanol (75 µL), 1 M NaOH (450 µL) and distilled water with proper shaking before adding the next solution. The mixture was mixed well and incubated at room temperature for 1 h. Thereafter, an aliquot (200 µL) was taken out in a 15 mL disposable tube and neutralized with 0.05 M citric acid (1 mL), followed by addition of 800 µL of $I_2$/KI solution [0.8 g iodine ($I_2$) and 8 g potassium iodide (KI) in 1 L of distilled water]. The mixture was mixed well and the volume was made up to 12 mL with distilled water. The absorbance was observed at 535 (reddish brown) and 620 (blue) nm for amylopectin and amylose, respectively.

### 3.3.6 Estimation of protein concentration

Total nitrogen was determined by combustion method (FP-528 Protein/Nitrogen Analyser, Leco Corporation, St Joseph, MI, USA) (Frimpong et al., 2009). Protein concentration was calculated by using the following formula (Karaca et al., 2011):

Protein (%) = % N × nitrogen to protein conversion factor (6.25 for chickpea seeds)…….....(3.1)

### 3.3.7 Statistical analysis

Shannon−Weaver diversity index (SDI) was calculated as

$$\text{SDI} = \left(-\sum_{i=1}^{n} P_i \times \log_e P_i\right) / \log_e n \quad \text{.....(3.2)}$$

where, n represents the total number of phenotypic classes, and $P_i$ is the proportion of total number of entries in the $i^{th}$ class (Bhattacharjee et al., 2007). Statistical analysis including box-plots, analysis of variance (using general linear model) and Pearson's correlation coefficients, was executed using MINITAB 14 statistical software (Minitab Inc., State College, PA, USA). Phenotypic classes were prepared by using MINITAB 14 statistical software (Minitab Inc., State College, PA, USA). Mixed model was used to calculate analysis of variance (ANOVA) in MINITAB 14 and resulted F values from ANOVA was utilized to determine the significance of the influences from genotype (G), environment (E) and their interaction (G × E). Covariance estimates of variance components were used to calculate heritability ($H^2$) as described by Singh (1993).

## 3.4 Results

### 3.4.1 Diversity among chickpea genotypes

Chickpea genotypes were collected from the center of origin, primary and secondary centers of distribution, and were analyzed for diversity with Shannon-Weaver diversity index (SDI). Although the center of origin has the least number of chickpea genotypes yet it showed the highest SDI for all seed constituent traits including seed weight (0.66), total starch (0.70), protein (0.66), and amylose (0.81) (Table 3.1).

### 3.4.2 Variation for seed constituent traits among chickpea genotypes

Chickpea genotypes were screened for one thousand seed weight (TSW), total starch, amylose and protein concentrations. In F 2011, TSW ranged from 90.9 to 306.7 g, 105.0 to 472.0 g and 167.0 to 248.1 g for desi, kabuli and pea-shaped genotypes, with mean values of 145.9, 242.2 and 184.7 g, respectively. Desi, kabuli and pea-shaped genotypes (mean values) varied from 67.8 to 334.9 g (149.5 g), 107.1 to 438.3 g (234.6 g), and 140.5 to 254.6 g (189.2 g) for TSW in F 2013. In controlled condition of G 2012, TSW ranged from 97.9 to 329.5 g (182.2 g), 114.8 to 382.3 g (259.4 g) and 165.1 to 269.7 g (213.5 g) in desi, kabuli and pea-shaped genotypes (mean values), respectively (Figure 3.1).

Total starch concentration varied significantly among desi, kabuli and pea-shaped genotypes (mean values) from 30.2 to 55.2 % (39.1 %), 38.3 to 51.3 % (49.0 %) and 39.8 to 45.5 % (41.9 %) during F 2011; whereas ranged from 34.2 to 48.7 % (40.9 %), 39.0 to 51.3 % (47.0 %.) and 43.5 to 47.1 % (42.3 %) during F 2013, respectively. During G 2012, total starch concentration varied from 31.6 to 60.0 %, 34.1 to 63.2 % and 40.1 to 55.9 %, with a mean of 43.7 %, 50.2 % and 45.3 % among desi, kabuli and pea-shaped genotypes, respectively (Figure 3.2).

Desi, kabuli and pea-shaped genotypes varied significantly for protein concentration (mean values) from 17.9 to 28.0 % (22.2 %), 20.8 to 27.8 % (23.4 %), and 20.4 to 24.2 % (22.4 %) during F 2011; while it ranged from 20.1 to 27.8 % (23.3 %), 19.6 to 28.0 % (24.1 %), and 23.1 to 25.2 % (24.2 %) during F 2013, respectively. In G 2012, protein concentration ranged from 13.7 to 24.5 % (17.4 %), 15.7 to 26.2 % (20.3 %), and 18.4 to 21.6 % (19.6 %) among desi, kabuli and pea-shaped genotypes, respectively (Figure 3.3).

Amylose concentration (mean values) ranged from 32.1 to 41.6 % (36.9 %), 33.0 to 39.6 % (35.6 %) and 32.5 to 42.4 % (36.2 %) during F 2011; whereas varied from 30.6 to 39.5 % (35.3 %), 28.8 to 37.4 % (34.2 %), and 34.4 to 38.8 % (35.9 %) during F 2013 among desi, kabuli and pea-shaped genotypes, respectively. During G 2012, amylose concentration (mean

**Table 3.1** Shannon−Weaver diversity index of chickpea genotypes categorized on the basis of their origin.

| Region | Number of chickpea genotypes | | | | SDI | | | |
|---|---|---|---|---|---|---|---|---|
| | Desi type | Kabuli type | Pea-shaped | Total | TSW | Total Starch | Protein | Amylose |
| Center of origin (Syria and Turkey) | 7 | 4 | 1 | 12 | 0.66 | 0.70 | 0.66 | 0.81 |
| Primary center of distribution (Israel and Iran) | 50 | 20 | 0 | 70 | 0.50 | 0.46 | 0.41 | 0.56 |
| Secondary center of distribution (other countries) | 122 | 23 | 7 | 152 | 0.41 | 0.38 | 0.46 | 0.50 |

Country of origin was unknown for three genotypes; therefore, Shannon−Weaver diversity index (SDI) was calculated for 234 chickpea genotypes.

TSW = one thousand seed weight.

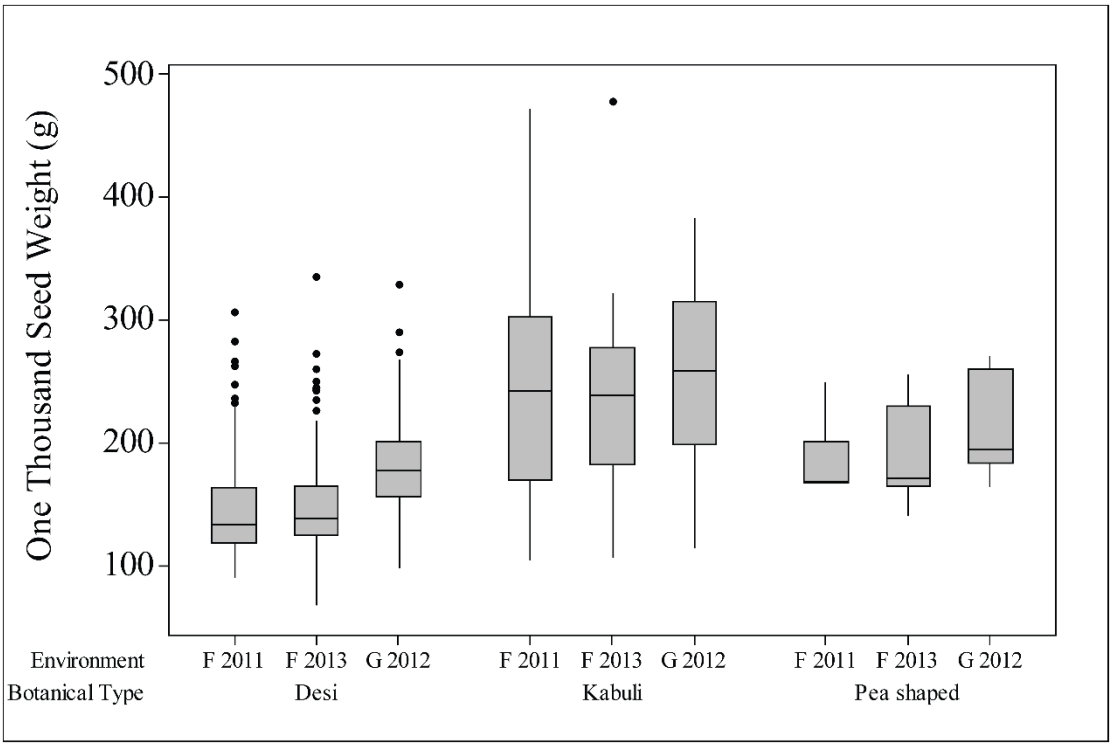


**Figure 3.1** Box plot analysis of one thousand seed weight for desi, kabuli and pea-shaped chickpea genotypes in different growing environments.
F 2011 and F 2013 represent the field trials of 2011 and 2013, respectively, while G 2012 represents the greenhouse trial in 2012. The upper and lower error bars represent the non-outlier range of the data set. The box represents the interquartile range (IQR), whereas the middle line shows the median value of the data set. The dark circles represent the outliers, calculated as the data points out of the 1.5 times the IQR.

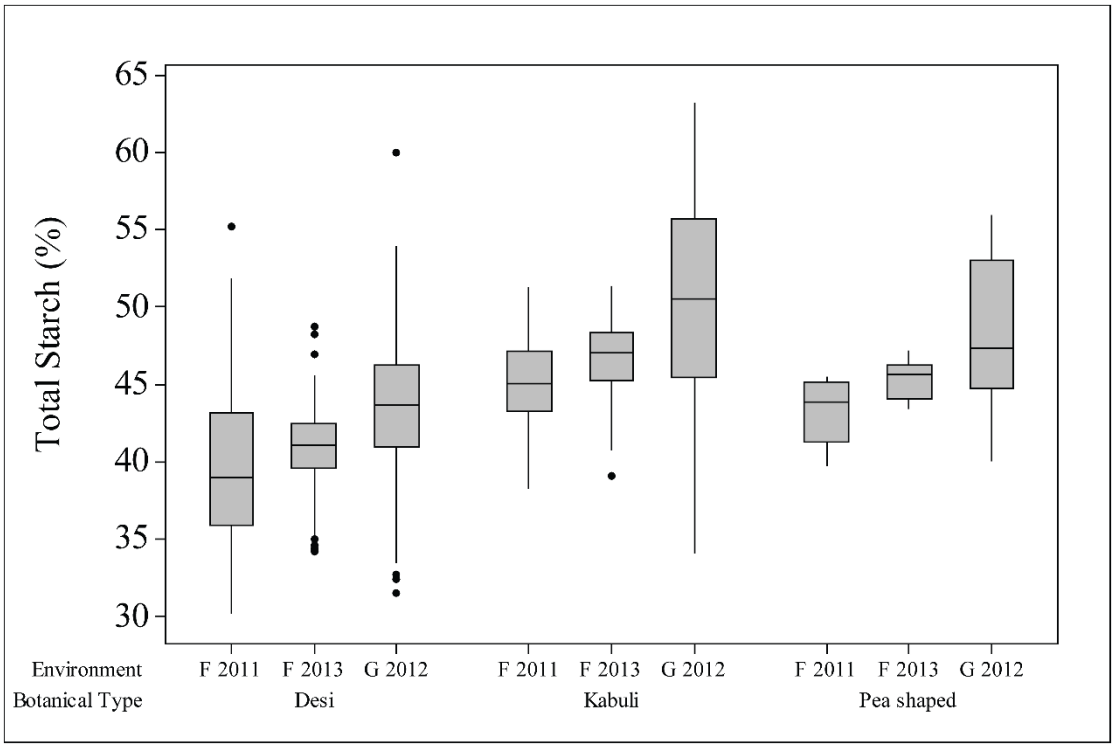


**Figure 3.2** Box plot analysis of total starch concentrations for desi, kabuli and pea-shaped chickpea genotypes in different growing environments.
F 2011 and F 2013 represent the field trials of 2011 and 2013, respectively, while G 2012 represents the greenhouse trial in 2012. The upper and lower error bars represent the non-outlier range of the data set. The box represents the interquartile range (IQR), whereas the middle line shows the median value of the data set. The diamonds represent the outliers, calculated as the data points out of the 1.5 times the IQR.

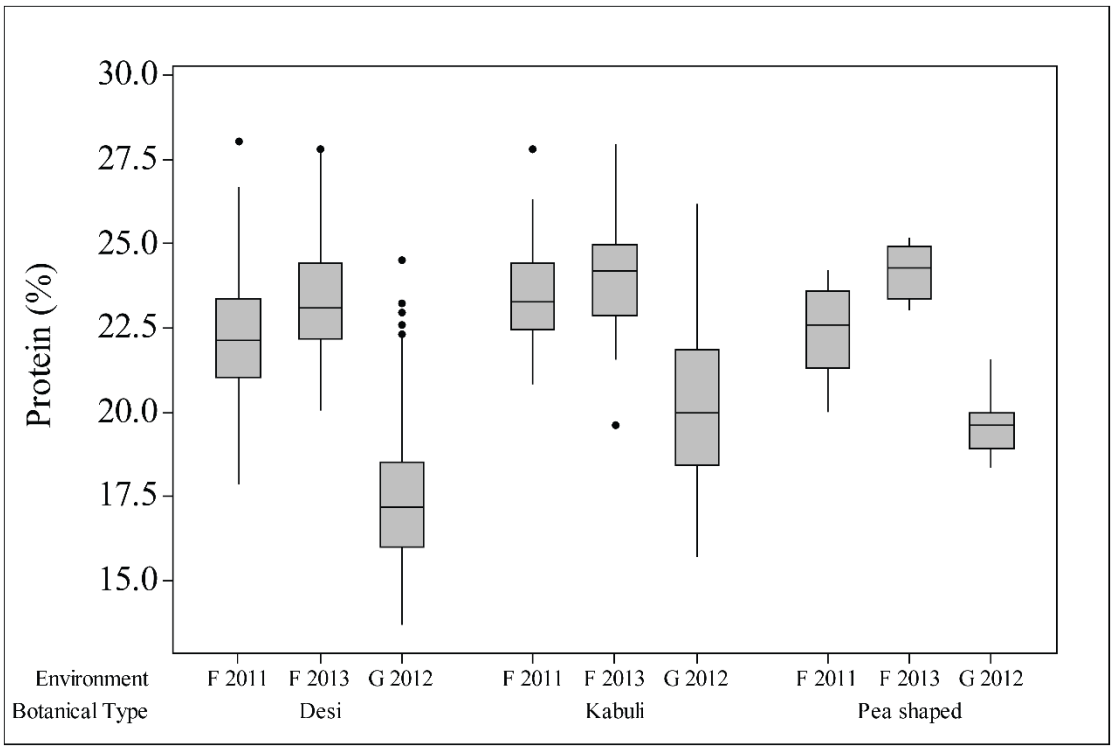


**Figure 3.3** Box plot analysis of protein concentrations for desi, kabuli and pea-shaped chickpea genotypes in different growing environments.
F 2011 and F 2013 represent the field trials of 2011 and 2013, respectively, while G 2012 represents the greenhouse trial in 2012. The upper and lower error bars represent the non-outlier range of the data set. The box represents the interquartile range (IQR), whereas the middle line shows the median value of the data set. The diamonds represent the outliers, calculated as the data points out of the 1.5 times the IQR.

values) varied from 27.1 to 41.4 % (32.3 %), 28.1 to 33.3 % (30.4 %), and 31.1 to 35.4 % (33.1 %) among desi, kabuli and pea-shaped genotypes, respectively (Figure 3.4).

ICC 16903 (desi type), ICC 7255 (kabuli type), and ICC 8350 (pea-shaped type) were selected for their consistent performance and higher values for protein (21.5 ± 4.4 %, 23.2 ± 2.2 %, and 22.6 ± 2.6 %), TSW (181.8 ± 15.6 g, 316.8 ± 30.3 g, and 251.8 ± 16.3 g), starch (44.4 ± 1.9 %, 51.2 ± 4.8 %, and 41.1 ± 5.7 %), and amylose (36.6 ± 1.6 %, 34.3 ± 3.9 %, and 35.5 %).

### 3.4.3 Effect of genotype × environment interaction on seed constituent traits

Analysis of variance (ANOVA) using mixed model established a significant ($P \leq 0.001$) effect of the interaction of genotype with environment (G×E) on TSW, total starch, amylose and protein concentrations in seeds of desi, kabuli and the pea-shaped chickpea genotypes (Table 3.2). CVs (%) ranged from 19.7 to 30.2 for TSW, from 7.7 to 12.8 for total starch, from 10.1 to 15.1 for protein, and from 8.3 to 9.1 for amylose, respectively (Table 3.2).

Broad sense heritability ($H^2$) has been classified in to three groups: high heritability (> 0.60), medium heritability (0.60 – 0.30), and low heritability (< 0.30) (Gangola et al., 2013). Chickpea genotypes showed the highest $H^2$ (0.70 to 0.74) for TSW followed by protein (0.16 to 0.29), amylose (0.11 to 0.17), and total starch (0.13 to 0.15) concentrations (Table 3.3).

### 3.4.4 Correlation among seed constituent traits

Correlation analysis was performed within and across environments for desi, kabuli and pea-shaped chickpea genotypes (Table 3.4). Desi genotypes showed a significant negative correlation ($r$ = -0.25 to -0.40; $P \leq 0.001$) between total starch and protein in all growth conditions; whereas significant positive correlation ($r$ = 0.26 to 0.47; $P \leq 0.001$) was observed between TSW and total starch during F 2013 and G 2012. In kabuli genotypes, TSW was positively correlated with starch ($r$ = 0.46 and 0.48; $P \leq 0.001$) and negatively with protein ($r$ = -0.35 and -0.42; $P \leq 0.05$ and 0.01, respectively) during F 2011 and F 2013; whereas a significant negative correlation ($r$ = -0.44 to -0.47; $P \leq 0.001$) was also found between total starch and protein during F 2011 and F 2013. In pea-shaped chickpea genotypes, TSW and amylose showed significant positive correlation ($r$ = 0.87; $P \leq 0.01$) that cannot be relied as only eight pea-shaped genotypes were analyzed in the present study. Overall correlation coefficients were also calculated among different seed constituent traits by pooling the data of all chickpea genotypes across all the environments (Table 3.4). Starch showed a significant positive correlation with TSW ($r$ = 0.52; $P \leq 0.001$) but negative with amylose ($r$ = -0.30; $P \leq$

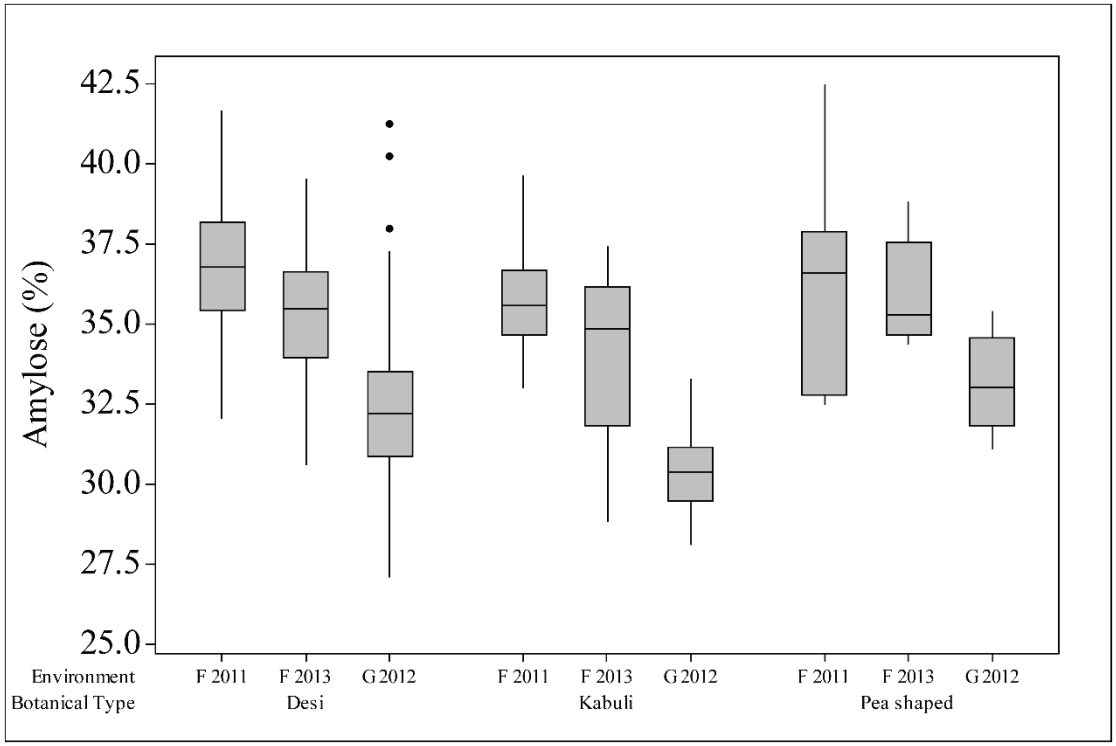


**Figure 3.4** Box plot analysis of amylose concentrations (% of total starch) for desi, kabuli and pea-shaped chickpea genotypes in different growing environments.
F 2011 and F 2013 represent the field trials of 2011 and 2013, respectively, while G 2012 represents the greenhouse trial in 2012. The upper and lower error bars represent the non-outlier range of the data set. The box represents the interquartile range (IQR), whereas the middle line shows the median value of the data set. The diamonds represent the outliers, calculated as the data points out of the 1.5 times the IQR.

**Table 3.2** Analysis of variance (ANOVA) with F values of chickpea genotypes for selected seed constituent traits.

| Type | Effect | numDF | F values | | | |
|---|---|---|---|---|---|---|
| | | | TSW | Total starch | Protein | Amylose |
| Desi | E | 2 | $31.40^{**}$ | $447.86^{***}$ | $170.69^{***}$ | $373.15^{***}$ |
| | G | 179 | $190.37^{***}$ | $51.46^{***}$ | $21.60^{***}$ | $16.30^{***}$ |
| | G × E | 358 | $20.94^{***}$ | $35.47^{***}$ | $13.64^{***}$ | $9.65^{***}$ |
| | CV (%) | | 27.05 | 12.84 | 15.08 | 8.28 |
| Kabuli | E | 2 | $1.01^{ns}$ | $332.63^{***}$ | $22.85^{**}$ | $170.14^{**}$ |
| | G | 48 | $163.11^{***}$ | $59.85^{***}$ | $25.18^{***}$ | $10.22^{***}$ |
| | G × E | 96 | $14.49^{***}$ | $38.08^{***}$ | $9.75^{***}$ | $6.85^{***}$ |
| | CV (%) | | 30.23 | 14.23 | 11.81 | 8.38 |
| Pea-shaped | E | 2 | $7.09^{ns}$ | $4.41^{ns}$ | $46.23^{**}$ | $180.67^{**}$ |
| | G | 7 | $115.44^{***}$ | $33.73^{***}$ | $15.53^{**}$ | $17.52^{**}$ |
| | G × E | 14 | $14.03^{***}$ | $22.43^{***}$ | $6.88^{**}$ | $12.40^{**}$ |
| | CV (%) | | 19.65 | 7.68 | 10.12 | 9.06 |
| Overall | E | 2 | $10.97^{*}$ | $672.64^{***}$ | $166.60^{**}$ | $820.32^{***}$ |
| | G | 236 | $252.65^{***}$ | $196.07^{***}$ | $29.15^{***}$ | $19.16^{***}$ |
| | G × E | 472 | $15.61^{***}$ | $49.99^{***}$ | $18.06^{***}$ | $13.81^{***}$ |
| | CV (%) | | 34.65 | 16.72 | 14.58 | 8.62 |

*, ** and *** are significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$; ns stands for non-significant. E, Environment; G, Genotype.

**Table 3.3** Estimates of variance components and broad-sense heritability ($H^2$) for selected seed constituent traits.

| Type | Variance component | TSW | Total starch | Protein | Amylose |
|---|---|---|---|---|---|
| Desi | $\sigma_g^2$ | $1.11\times10^3\pm1.32\times10^2$ | 1.18±0.75 | 0.85±0.17 | 0.68±0.27 |
| | $\sigma_{gl}^2$ | $3.92\times10^2\pm30.81$ | 6.54±1.19 | 3.45±0.28 | 2.81±0.18 |
| | $\sigma_e^2$ | 78.72±2.77 | 1.39±0.05 | 1.02±0.04 | 0.53±0.02 |
| | $\sigma_p^2$ | $1.58\times10^3$ | 9.11 | 5.32 | 4.02 |
| | $H^2$ | 0.70 | 0.13 | 0.16 | 0.17 |
| Kabuli | $\sigma_g^2$ | $3.94\times10^3\pm8.84\times10^2$ | 4.29±2.64 | 1.25±0.43 | 0.62±0.25 |
| | $\sigma_{gl}^2$ | $1.07\times10^3\pm1.66\times10^2$ | 21.91±3.25 | 2.12±0.34 | 2.60±0.42 |
| | $\sigma_e^2$ | $3.18\times10^2\pm21.51$ | 2.35±0.16 | 0.97±0.07 | 1.18±0.08 |
| | $\sigma_p^2$ | $5.33\times10^3$ | 28.55 | 4.34 | 4.40 |
| | $H^2$ | 0.74 | 0.15 | 0.29 | 0.14 |
| Pea-shaped | $\sigma_g^2$ | $1.05\times10^3\pm6.22\times10^2$ | 1.58±2.78 | 0.34±0.14 | 0.88±0.22 |
| | $\sigma_{gl}^2$ | $3.00\times10^2\pm1.22\times10^2$ | 8.99±3.56 | 0.68±0.30 | 5.89±1.90 |
| | $\sigma_e^2$ | 9.21±15.68 | 1.68±0.29 | 0.47±0.08 | 1.10±0.18 |
| | $\sigma_p^2$ | $1.36\times10^3$ | 12.25 | 1.49 | 7.87 |
| | $H^2$ | 0.73 | 0.13 | 0.23 | 0.11 |

$\sigma_g^2$ = genotypic variance; $\sigma_{gl}^2$ = genotype × location interaction variance; $\sigma_e^2$ = error variance; $\sigma_p^2$ = phenotypic variance.

0.001) and protein ($r$ = -0.20; $P \leq 0.001$). Amylose was positively correlated with protein ($r$ = 0.38; $P \leq 0.001$) and negatively with TSW ($r$ = -0.20; $P \leq 0.001$). Protein showed a significant negative correlation with TSW ($r$ = -0.11; $P \leq 0.001$) (Table 3.4).

### 3.5 Discussion

To develop breeding strategies for chickpea seed quality improvement, important traits such as seed weight, total starch, amylose and protein were characterized in 237 chickpea genotypes including 180 desi, 49 kabuli and 8 pea shaped, grown in replicated trials over three consecutive years in field (2011 and 2013) and greenhouse (2012). Kabuli genotypes showed the highest TSW, total starch and protein concentrations followed by pea-shaped and desi genotypes. However, desi genotypes showed higher amylose concentration compared to others. The values of TSW, total starch and protein concentrations concur with the results of previous studies. In previous studies, desi/kabuli genotypes ranged from 132 to 339/150 to 449 g, 38.5 to 51.0/39.0 to 57.2 %, and 18.0 to 28.0/17.1 to 31.0 % for TSW, total starch and protein concentrations, respectively (Saini and Knights, 1984; Khan et al., 1995; Maheri-Sis et al., 2008; Frimpong et al., 2009; Sharma et al., 2013b). These previous studies also support the higher diversity of kabuli genotypes for the seed constituent traits. Upadhyaya et al. (2002) also identified kabuli genotypes with the highest TSW ($P \leq 0.001$) followed by desi and pea-shaped genotypes in a core collection of 1,965 chickpea genotypes. However, amylose concentration in the present study was relatively higher compared to 26.4 – 27.6 % and 27.1 – 28.0 % in desi and kabuli genotypes, respectively as reported earlier (Frimpong et al., 2009). It could be due to difference in population size, genotypes, environment and amylose determination method. Optimum growth conditions support starch biosynthesis in seeds (Famera et al., 2015); therefore, G 2012 grown chickpea genotypes showed higher starch accumulation in seeds compared to F 2011 and 2013. However, wet environment has been associated with increased α-amylase activity and reduced starch accumulation in wheat grains (Famera et al., 2015) that concur with reduced starch accumulation in F 2011 grown chickpea genotypes.

The estimation of G × E effects showed a significant influence of the growing environments on the selected seed constituent traits. These results concur with the conclusions of Frimpong et al. (2009) showing a significant effect ($P \leq 0.05$) of genotype × location on TSW, total starch, amylose and protein concentrations in seven desi and nine kabuli chickpea genotypes. G, E and G×E also showed a significant ($P \leq 0.001$) effect on soluble sugars

**Table 3.4** Correlations among selected seed constituent traits.

| Seed trait | Environment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F 2011 | | | F 2013 | | | G 2012 | | |
| | TSW | Starch | Amylose | TSW | Starch | Amylose | TSW | Starch | Amylose |
| | | | | **Desi genotypes** | | | | | |
| Starch | $0.08^{ns}$ | | | $0.47^{***}$ | | | $0.26^{***}$ | | |
| Amylose | $-0.05^{ns}$ | $-0.12^{ns}$ | | $0.12^{ns}$ | $0.07^{ns}$ | | $0.18^{*}$ | $0.01^{ns}$ | |
| Protein | $-0.01^{ns}$ | $-0.25^{***}$ | $-0.28^{***}$ | $-0.06^{ns}$ | $-0.40^{***}$ | $0.20^{*}$ | $-0.05^{ns}$ | $-0.31^{***}$ | $0.04^{ns}$ |
| | | | | **Kabuli genotypes** | | | | | |
| Starch | $0.46^{***}$ | | | $0.48^{***}$ | | | $0.26^{ns}$ | | |
| Amylose | $0.03^{ns}$ | $0.15^{ns}$ | | $0.23^{ns}$ | $0.04^{ns}$ | | $0.03^{ns}$ | $0.07^{ns}$ | |
| Protein | $-0.35^{*}$ | $-0.44^{***}$ | $0.05^{ns}$ | $-0.42^{**}$ | $-0.47^{***}$ | $-0.19^{ns}$ | $-0.25^{ns}$ | $-0.05^{ns}$ | $0.07^{ns}$ |
| | | | | **Pea-shaped genotypes** | | | | | |
| Starch | $-0.51^{ns}$ | | | $-0.43^{ns}$ | | | $-0.32^{ns}$ | | |
| Amylose | $0.09^{ns}$ | $-0.11^{ns}$ | | $0.87^{**}$ | $-0.23^{ns}$ | | $-0.60^{ns}$ | $0.36^{ns}$ | |
| Protein | $0.05^{ns}$ | $0.03^{ns}$ | $0.02^{ns}$ | $-0.05^{ns}$ | $0.34^{ns}$ | $-0.10^{ns}$ | $-0.02^{ns}$ | $0.57^{ns}$ | $0.09^{ns}$ |

**Overall**

| | TSW | Starch | Amylose |
|---|---|---|---|
| Starch | 0.52*** | | |
| Amylose | -0.20*** | -0.30*** | |
| Protein | -0.11*** | -0.20*** | 0.38*** |

***, ** and * are significant at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$, respectively; $^{ns}$ stands for non-significant.

concentration in chickpea seeds (Gangola et al., 2013).

Seed weight as an extrinsic seed quality trait was recorded with high $H^2$ (0.60 to 0.99) and repeatability (0.91 to 0.94) across genotypes and environments in previous studies (Saleem et al., 2002; Tuba Bicer and Sakar, 2008; Frimpong et al., 2009; Parameshwarappa et al., 2012; Mallu et al., 2014). In contrast to present study, total starch (0.67 – 0.90), amylose (0.34 – 0.49) and protein (0.37 – 0.86) concentrations in seeds of other legumes showed moderate to high $H^2$ (Guzhov and Gneim, 1981; Lawn and Rebetzke, 2006; Burstin et al., 2007; Hood-Niefer et al., 2012; Gerrano et al., 2015). These lower $H^2$ values in present study can be attributed to distinct growth conditions during F 2011, F 2013 and G 2012, and inconsistent performance of genotypes across these environments. A significant impact of G×E supports the complex regulation of seed constituent traits especially for total starch, amylose and protein concentrations in chickpea seeds resulting in relatively low heritability of these traits in the present study.

The negative correlation between starch and protein is supported by a recent study (Gaur et al., 2016). Starch and protein compete for carbon source or photosynthate (Jenner et al., 1991; Rolletschek et al., 2002); therefore, showed a negative correlation in chickpea genotypes. Additionally, a higher activity of α-amylase in wet environment (Famera et al., 2015) may catabolize starch (especially amylopectin), accumulate soluble sugars and increased water influx that can accumulate amino acids to synthesize more protein (Rolletschek et al., 2002), which may also explain the positive correlation between protein and amylose. Although, independent selection of genotypes for starch and protein concentration has been suggested, yet their correlation and its effect on yield cannot be ignored (Gaur et al., 2016). Therefore, more detailed investigation is required to understand the correlation between total starch and protein. The correlation among seed constituent traits are also supported by studies in chickpea (Frimpong et al., 2009) and lentil (*Lens culinaris* Medik.; Tahir et al., 2011) .

SDI estimates showed the highest values for all of the selected seed constituent traits, *i.e.* 0.66 for TSW, 0.70 for total starch, 0.66 for protein and 0.81 for amylose. Center of origin includes domesticated species and wild or weedy relatives of a gene pool, thus represents maximum genetic diversity (Engels et al., 2006; Gangola et al., 2013). Therefore, this region showed the maximum diversity for seed constituent traits. Presence of higher genetic diversity in the chickpea collection also supports its usefulness in studying natural variation for seed constituent traits.

# CHAPTER 4. GENOME-WIDE ASSOCIATION STUDY REVEALS MARKER-TRAIT ASSOCIATION FOR SELECTED SEED CONSTITUENT TRAITS IN CHICKPEA (*Cicer arietinum* L.)

## 4.1 Study 2*

The second study utilized a chickpea composite collection to study the natural variation in chickpea seed composition traits such as thousand seed weight, protein, starch and amylose concentration. Diversity Array Technology (DArT) was used to study genetic variation and identify marker trait association (MTA) for selected seed constituent traits.

* Wang, R., Gangola, M. P., Khedikar, Y. P., Jaiswal, S., Gaur, P. M., Båga, M., and Chibbar, R. N. 2016. Genome-wed association study reveals marker-trait association for selected seed constituent traits in chickpea (*Cicer arietinum* L.) (to be submitted).

## 4.2 Introduction

Chickpea (*Cicer arietinum* L.) is an annual self-pollinating member of the Fabaceae family. It is a diploid species with a basic chromosomal number of eight (2n = 2x = 16) and a genome size of 740 mbp (Varshney et al., 2013). The annual production of chickpea was 14.2 million tonnes cultivated over 14.8 million hectare area during 2014 that makes chickpea globally the second most important pulse crop (FAOSTAT, 2014). Chickpea is classified into two botanical groups: desi (purple flower and small, dark, angular seeds) and kabuli (white flower and large, cream-colored seeds) types that are mainly grown in semiarid tropical and temperate regions of the world, respectively. The global annual production of desi chickpea is about four times compared to that of kabuli type (Thudi et al., 2014). Chickpea is a very good source of carbohydrates and protein contributing 45 – 60 % and up to 30 % to dry seed mass, respectively (Zia-Ul-Haq et al., 2007; Jukanti et al., 2012). Lipid concentration ranges from 6 to 9 %, of which 85 % is composed of unsaturated fatty acids (Jukanti et al., 2012). Chickpea, rich in lysine and deficient in methionine amino acids, makes an excellent complementary food source to cereals which lacks lysine but are rich in methionine (Wood and Grusak, 2007). Amylose constitutes about 30 – 40 % of starch in chickpea seeds which is significantly higher than that in cereals (25 % in wheat) (Chibbar et al., 2010). Higher concentrations of poly-unsaturated fatty acid (66 % of total fatty acids) and dietary fibers (18 – 22 %) in chickpea seeds have also been attributed to promote human and animal health (Mathers, 2002; Duranti, 2006; Pittaway et al., 2007; Yang et al., 2007).

Chickpea breeding programs have mainly ensued to increase the yield by improving either valuable agronomic traits (Cho et al., 2002; Cobos et al., 2009) or resistance/tolerance to biotic/abiotic stresses (Tar'an et al., 2007; Sabbavarapu et al., 2013; Patil et al., 2014). Consequently, yield has been increased from 649.0 kg/ha in 1965 to 959.5 kg/ha in 2014 (FAOSTAT, 2014). However, breeding for nutritional quality improvement has rarely been attempted in chickpea (Gaur et al., 2007). Chickpea has a narrow genetic diversity attributed to its monophyletic origin, confined distribution of wild progenitor, change in sowing time and adoption of elite cultivars (Abbo et al., 2003). Therefore, new genetic resources need to be identified and utilized in chickpea breeding programs to improve its nutritional quality.

To identify genomic regions affecting chickpea seed quality, two strategies, bi-parental mapping population based linkage analysis and linkage disequilibrium (LD) based association study, can be followed. Bi-parental linkage mapping requires highly pedigreed mapping population with adequate recombination events to detect quantitative trait locus (QTL). However, developing such population is labor and time intensive compared to association

mapping which utilizes natural population arising from historical recombination events and yields a higher resolution than linkage analysis (Myles et al., 2009). Association mapping is also capable of identifying more alleles and marker-trait associations (MTA) in various traits simultaneously with statistical evaluation of measurable variation in the mapping population (Yu and Buckler, 2006; Zhu et al., 2008). Therefore, association mapping combined with high throughput genotyping technologies, has been extensively used to detect novel loci associated with simple to complex traits in several crops (Abdurakhmonov and Abdukarimov, 2008).

Diversity array technology (DArT) is a high throughput marker system that is rapid, highly reproducible and cost effective compared to conventional PCR based genotyping techniques such as RAPD and AFLP (Semagn et al., 2006a). The DArT marker system can assay a large number of loci in more than hundreds of accessions thus providing better illustration of genetic relationship in a germplasm collection (Jaccoud et al., 2001; Wenzl et al., 2004; Schouten et al., 2012). DArT markers, have been successfully used in QTL analysis for yield/grain quality in winter wheat (Tadesse et al., 2015) and tuber starch/leaf sucrose contents in diploid potato (Sliwka et al., 2016).

In the present study a chickpea composite germplasm collection was characterized for selected seed composition traits in a multi-environment test. DArT marker analyses identified MTAs for 1000-seed weight, total starch, protein and amylose concentration in the chickpea composite germplasm collection.

## 4.3 Material and methods

### 4.3.1 Plant material

A composite collection, designed for seed constituent traits, of 168 chickpea genotypes, was procured from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, Patancheru, India). The collection includes both desi (115) and kabuli (53) chickpea genotypes (composed of breeding/advanced lines and landraces with beneficial traits) collected from eight regions of the world for the purpose of seed quality evaluation (Appendix 5). All of the chickpea accessions were grown in two field trials during 2008-2009 (F 2009) and 2009-2010 (F 2010), with one greenhouse trial during 2010 (G 2010). Field and greenhouse experiments were performed at ICRISAT (17 ° 53 ′ N latitude, 78 ° 27 ′ E longitude and 545 m altitude) and the agriculture greenhouse of University of Saskatchewan (52 ° 07 ′ N latitude, 106 ° 38 ′ W longitude and 481.5 m altitude, Saskatoon, SK, Canada), respectively. Field experiments were performed in a randomized complete block design (25 cm spacing between rows) from October to mid-March at ICRISAT in two biological replications whereas G 2012

was performed from March to July, 2010. The daily mean minimum and maximum temperatures were 15.0 and 31.1 °C, respectively for F 2009 with an average of 8.9 h of bright sunshine, ~352.1 μM/$m^2$/s of solar radiation and 6.5 mm of precipitation. For F 2010, the mean daily minimum and maximum temperatures were 16.2 and 30.0 °C, respectively with an average of 8.1 h of bright sunshine, ~333.4 μM/$m^2$/s of solar radiation and 18.7 mm of precipitation. In controlled conditions (G 2010), the average daily minimum and maximum temperatures were 18 and 23 °C with an 18 h photoperiod and 385 μM/$m^2$/s of photosynthetically active radiation. Mature plants were harvested, threshed and seeds were stored at room temperature. All genotypes were grown in two blocks. In each block, 50 seeds of one genotype were seeded in a single row. When harvested, all seeds from the 50 individual plants of the same genotype were bulked. For each genotype, the selected seed constituent traits were analyzed two times for each block, giving two biological replicates for each block. For each biological replicate in each block, two technical replicates were analyzed, giving eight technical replicates in total. Field trials of 2009 and 2010 were carried out by researchers and employees at ICRISAT and seeds were sent to the University of Saskatchewan for analysis.

### 4.3.2 Phenotypic evaluation of selected seed constituent traits

One-thousand seed weight was measured using electronic seed counter (Seedburo Equipment Co., Chicago, IL, USA). Total nitrogen was estimated by combustion method (FP-528 Protein/Nitrogen Analyser, Leco Corporation, St Joseph, MI, USA). Total nitrogen was multiplied by nitrogen to protein conversion factor (6.25 for chickpea seeds) to obtain protein concentration (Karaca et al., 2011). Total starch concentration was determined by an enzymatic hydrolysis method using a commercial kit (Megazyme International Ireland Ltd., Wicklow, Ireland). For amylose determination, starch was extracted using a modified method (Peng et al., 1999) including cesium chloride density gradient centrifugation (Asare et al., 2011). The purified starch was used to determine amylose concentration using iodine based method with some modifications (Mahmooda et al., 2007); therefore, it is expressed as percentage of total starch.

### 4.3.3 Statistical analysis

Shannon−Weaver diversity index (SDI) was calculated to determine diversity in each geographical region using following formula (Gangola et al., 2013):

$$\text{SDI} = \frac{-\sum_{i=1}^{n} P_i \times log_e P_i}{log_e n} \quad \text{.....(4.1)}$$

where, $n$ represents the total number of phenotypic classes, and $P_i$ is the proportion of total number of entries in the $i^{th}$ class. Phenotypic classes were prepared by using MINITAB 14 statistical software (Minitab Inc., State College, PA, USA). Mixed model was used to calculate analysis of variance (ANOVA) in MINITAB 14. Significance of the influences from genotype (G), environment (E) and their interaction (G × E) was determined based on F values from the ANOVA. Covariance estimates of variance components were used to calculate heritability ($H^2$) as described by Singh et al. (1993).

### 4.3.4 DNA extraction and genotyping with DArT

Three seeds from each chickpea accession were grown in the greenhouse at the University of Saskatchewan. Young leaves (a composite sample of three leaves from three different plants) were collected two weeks after sowing, immediately frozen into liquid nitrogen and stored at -80 °C. The frozen leaves were used to extract genomic DNA using CTAB DNA extraction protocol as described (Cuc et al., 2008). DNA integrity and concentration were determined by agarose (0.8 %) gel electrophoresis and NanoDrop 8000 (Thermo Fisher Scientific, ON, Canada), respectively. DNA concentrations of all the samples were normalized to 100 ng/µl. Genotyping was performed using chickpea DArT arrays at Diversity Arrays Technology Pty. Ltd. (Bruce, ACT2617 Australia; http://www.diversityarrays.com).

### 4.3.5 DArT marker quality analysis

The polymorphism information content (PIC) was calculated for each DArT marker using the following formula (Anderson et al., 1993):

$$PIC = 1 - \sum P_i^2 \quad \text{……..……..…(4.2)}$$

where, $P_i$ is the frequency of the $i^{th}$ allele in the examined genotypes. DArT markers were also evaluated on the basis of their P value (%), reproducibility (%), discordance and call rate (%) as described by Van Schalkwyk et al. (2012).

### 4.3.6 Population structure analysis

Population structure of 168 chickpea genotypes in the composite collection was determined using DArT markers in fastSTRUCTURE version 1.0 (Raj et al., 2014) and STRUCTURE version 2.3.4 (Pritchard et al., 2000). In both software packages, $K$ (number of sub-populations) values from 1 to 10 were tested. For STRUCTURE version 2.3.4, length of burn-in and the Markov chain Monte Carlo (MCMC) were set to 10,000 each and 20 runs were

performed to determine the variation in data likelihood at each *K*. The output files from STRUCTURE were analyzed to determine population structure using the *ΔK* method (Evanno et al., 2005) available in Structure Harvester version 0.6.94 (http://taylor0.biology.ucla.edu/structureHarvester/; Earl and Vonholdt, 2012).

DArT markers were used to calculate Jaccard's dissimilarity matrix using DARwin version 6.0.12 (Perrier et al., 2003). Jaccard's dissimilarity matrix was also utilized for principal co-ordinate analysis (PCoA) using DARwin version 6.0.12 to visualize genetic groups within the chickpea germplasm collection. A phylogenetic tree of the 168 chickpea accessions was graphically illustrated using UPGMA method in TASSEL version 5.2.20 (Bradbury et al., 2007).

#### 4.3.7 Analysis of molecular variance (AMOVA)

AMOVA was performed using the DArT markers to assess the variation among and within populations using GenAlEx version 6.5 (Peakall and Smouse, 2012). The significance of variance component was tested using 1000 permutations. AMOVA was conducted on sub-populations inferred by population structure analysis.

#### 4.3.8 Association analysis

The mixed linear model (MLM) approach was implemented to identify significant marker-trait associations using TASSEL version 5.2.20 that requires marker data, Q-matrix derived from fastSTRUCTURE, phenotypic data and kinship among chickpea accessions (Zhang et al., 2010b). Marker data were filtered using the "sites" option by default setting for minimum genotype count and to remove minor allele frequency (MAF). Marker data was filtered for a minimum count of 126 (75 %) of 168 accessions and markers having MAF < 0.5 % were removed from the data. MLM was performed using kinships to control type I errors. Bonferroni corrections ($P \leq 0.01$) were employed to identify significant markers in MLM. Markers showing $P \leq 0.05$ in MLM were considered as significantly associated markers with the traits under study. To identify trait-associated markers, the phenotypic variation ($R^2$) was calculated using the MLM procedure in TASSEL.

### 4.4 Results

#### 4.4.1 Characterization of variation in major seed constituent traits

Kabuli genotypes (97.0 – 528.0, 107.0 – 579.0 and 85.3 – 505.2 g) showed significantly ($P < 0.05$) higher TSW compared to desi types (89.0 – 380.0, 110.0 – 380.0 and 113.0 – 362.6

g) during F 2009, F 2010 and G 2010, respectively (Figure 4.1a). Desi genotype seeds contained 15.1 – 27.8 % (19.1 %), 17.1 – 30.1 % (23.8 %) and 15.3 – 25.3 % (19.5 %) of protein (mean values) while, it ranged 16.2 – 23.2 % (19.3 %), 18.2 – 31.6 % (25.1 %) and 14.8 – 24.7 % (19.4 %) among kabuli genotypes during F 2009, F 2010 and G 2010, respectively (Figure 4.1b). Total starch concentration ranged from 33.7 to 47.4 %, 31.3 to 43.3 % and 31.3 to 43.3 % among desi genotypes with average values of 39.6 %, 36.1 % and 38.4 % whereas it varied from 36.5 to 53.8 %, 33.0 to 48.2 % and 34.0 to 50.5 % in kabuli types with mean values of 44.6, 40.8 and 43.3 % during F 2009, F 2010 and G 2010, respectively (Figure 4.1c). In desi genotypes, amylose constituted 26.8 – 38.3 %, 30.0 – 38.1 % and 29.1 – 35.4 % of total starch with mean values of 31.6, 34.3 and 31.2 % whereas in kabuli genotypes, total starch contained about 28.0 – 45.1 %, 29.7 – 37.8 % and 28.7 – 33.9 % of amylose with average values of 31.3, 34.2 and 31.5 % during F 2009, F 2010 and G 2010, respectively (Figure 4.1d). Desi and kabuli chickpea genotypes with consistent performance and higher values in different environments were identified for the selected seed constituent traits separately (Table 4.1). Three genotypes, ICC 4958 (desi type), ICC 93954 (desi type), and ICC 8261 (kabuli type), exhibited balanced proportion of protein (20.5 ± 3.8, 20.0 ± 1.7, and 22.3 ± 2.1 %), total starch (43.4 ± 5.5, 44.8 ± 1.5, and 43.9 ± 6.5 %) and amylose (33.7 ± 1.5, 33.4 ± 1.4, and 33.5 ± 2.0 % of total starch), with TSW (342.6 ± 32.5, 315.7 ± 25.5, and 335.3 ± 42.6 g) towards the high concentrations, respectively.

### 4.4.2 Impact of genotype, environment and their interaction on chickpea seed constituent traits

Analysis of variance (ANOVA) established a significant ($P < 0.001$) effect of genotypes (G), environments (E) and their interaction (G × E) on selected seed constituent traits in both desi and kabuli chickpea types (Table 4.2). TSW (0.87 and 0.72) was identified with the highest broad sense heritability ($H^2$) followed by total starch (0.30 and 0.48), protein (0.34 and 0.17) and amylose (0.12 and 0.17) in chickpea genotypes (desi and kabuli types, respectively) (Table 4.3).

### 4.4.3 Correlation analysis and SDI for selected seed constituent traits

In desi genotypes, TSW was positively correlated with total starch but negatively with amylose and protein significant at $P < 0.01$. Total starch showed a significant ($P < 0.01$) negative correlation with amylose and protein; however, amylose and protein were positively correlated in desi genotypes significant at $P < 0.01$ (Table 4.4). Kabuli genotypes showed the

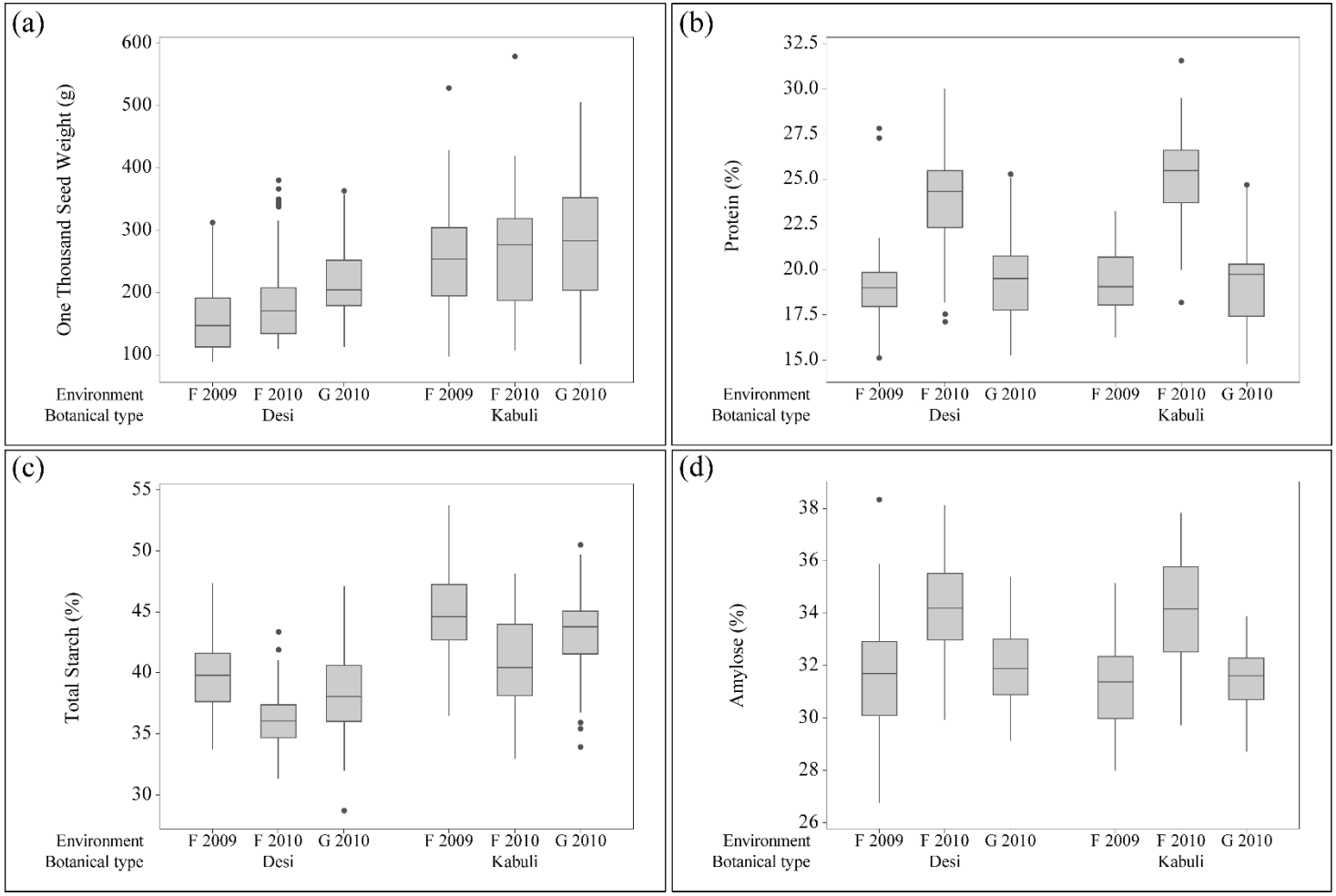


**Figure 4.1** Box plot analysis for **(a)** one thousand seed weight (g), **(b)** protein (%), **(c)** total starch (%), and **(d)** amylose (% of total starch) in desi and kabuli chickpea genotypes grown in different environments.
F 2009 and F 2010 represent the field trials of 2008-2009 and 2009-2010, respectively whereas, G 2010 represents the greenhouse trial in 2010. The upper and lower error bars represent the non-outlier range of the data set. The box represents the interquartile range (IQR), whereas the middle line shows the median value of the data set. The dark circles represent the outliers, calculated as the data points out of the 1.5 times the IQR.

**Table 4.1** Desi and kabuli chickpea genotypes with high values for selected seed constituent traits.

| Trait | Genotype | Botanical type | Value for phenotype (Mean±SD) |
|---|---|---|---|
| One-thousand seed weight (g) | ICC 4958 | Desi | 342.6±34.5 |
| | ICCV 07108 | Desi | 330.8±34.5 |
| | ICCV 94916-4 | Desi | 325.4±24.5 |
| | ICCV 94916-8 | Desi | 316.1±24.4 |
| | ICCV 98902 | Desi | 332.4±31.3 |
| | ICC 16774 | Kabuli | 394.9±30.2 |
| | ICC 17109 | Kabuli | 537.4±37.8 |
| | ICCV 07313 | Kabuli | 412.9±19.4 |
| Protein concentration (%) | ICC 5912 | Desi | 27.1±1.9 |
| | ICC 8397 | Desi | 27.5±2.4 |
| | ICC 4861 | Kabuli | 23.1±3.5 |
| | ICC 5116 | Kabuli | 24.3±6.3 |
| | ICC 5270 | Kabuli | 24.8±4.1 |
| Total starch concentration (%) | ICCV 93954 | Desi | 43.5±0.4 |
| | ICCV 98901 | Desi | 41.6±1.0 |
| | ICCV 98904 | Desi | 41.6±1.1 |
| | ICCV 06302 | Kabuli | 47.0±1.5 |
| | ICCV 07313 | Kabuli | 46.4±0.5 |
| | ICCV 91302 | Kabuli | 49.0±1.3 |
| Amylose concentration (% of total starch) | ICC 14456 | Desi | 34.4±0.8 |
| | ICC 14497 | Desi | 34.4±1.4 |
| | ICC 14592 | Desi | 34.2±0.2 |
| | ICC 7292 | Kabuli | 34.9±1.7 |
| | ICC 8273 | Kabuli | 34.6±2.4 |

**Table 4.2** Analysis of variance (ANOVA) with F values for selected seed constituent traits.

| Type | Effect | numDF | F values | | | |
|---|---|---|---|---|---|---|
| | | | TSW | Total starch | Protein | Amylose |
| Desi | E | 2 | 55.32** | 292.22*** | 1814.38*** | 629.51*** |
| | G | 114 | 210.74*** | 32.08*** | 84.36*** | 12.36*** |
| | G × E | 228 | 6.73*** | 12.47*** | 31.29*** | 8.08*** |
| | CV (%) | | 33.99 | 8.84 | 18.61 | 9.92 |
| Kabuli | E | 2 | 9.96* | 476.80*** | 941.76*** | 194.44** |
| | G | 52 | 68.34*** | 49.65*** | 56.01*** | 5.82*** |
| | G × E | 104 | 5.32*** | 11.05*** | 34.11*** | 2.44*** |
| | CV (%) | | 36.39 | 9.00 | 20.31 | 10.12 |
| Overall | E | 2 | 142.19** | 352.32*** | 1432.52*** | 599.92*** |
| | G | 167 | 124.81*** | 75.01*** | 73.57*** | 7.01*** |
| | G × E | 334 | 5.90*** | 11.93*** | 32.61*** | 5.69*** |
| | CV (%) | | 39.05 | 10.67 | 19.19 | 9.99 |

*, ** and *** are significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$; ns stands for non-significant. E, Environment; G, Genotype.

**Table 4.3** Estimates of variance components and broad-sense heritability ($H^2$) for selected seed constituent traits.

| Type | Variance component | TSW | Total starch | Protein | Amylose |
|---|---|---|---|---|---|
| Desi | $\sigma_g^2$ | $3.04\times10^3\pm4.06\times10^2$ | 2.27±0.50 | 1.69±0.36 | 0.50±0.33 |
| | $\sigma_{gl}^2$ | $2.57\times10^2\pm27.57$ | 3.99±0.40 | 2.90±0.27 | 2.41±0.20 |
| | $\sigma_e^2$ | $1.79\times10^2\pm7.68$ | 1.39±0.06 | 0.38±0.02 | 1.16±0.05 |
| | $\sigma_p^2$ | $3.48\times10^3$ | 7.65 | 4.97 | 4.07 |
| | $H^2$ | 0.87 | 0.30 | 0.34 | 0.12 |
| Kabuli | $\sigma_g^2$ | $6.44\times10^3\pm1.33\times10^3$ | 5.17±1.28 | 0.96±0.51 | 0.96±0.33 |
| | $\sigma_{gl}^2$ | $1.33\times10^3\pm2.21\times10^2$ | 4.04±0.60 | 4.36±0.61 | 1.22±0.28 |
| | $\sigma_e^2$ | $1.23\times10^3\pm77.51$ | 1.61±0.10 | 0.53±0.03 | 3.40±0.21 |
| | $\sigma_p^2$ | $9.00\times10^3$ | 10.82 | 5.85 | 5.58 |
| | $H^2$ | 0.72 | 0.48 | 0.17 | 0.17 |

$\sigma_g^2$ = genotypic variance; $\sigma_{gl}^2$ = genotype × location interaction variance; $\sigma_e^2$ = error variance; $\sigma_p^2$ = phenotypic variance.

similar correlation among selected seed constituent traits as observed in desi types except for amylose showing similar but insignificant correlation to TSW, total starch and protein (Table 4.4). North African genotypes (0.65) showed the maximum Shannon-Weaver diversity index (SDI) for TSW. Genotypes procured from Sub-Saharan Africa had the highest SDI for total starch (0.65) and protein (0.63). European genotypes were observed with the highest SDI of 0.61 for amylose (Table 4.5).

### 4.4.4 Quality assessment of DArT markers

The genomic evaluation of 168 chickpea genotypes with DArT markers revealed 380 out of 15,360 markers with highly informative polymorphism which was measured for each DArT marker by PIC (Figure 4.2). PIC values for 380 DArT markers ranged from 0.01 to 0.50 with a mean of 0.16. A large proportion of markers, 222 out of 380 (58.4 %), had PIC values less than 0.10. About 12.9 % markers were observed with PIC values ranging from 0.10 to 0.20. DArT markers having PIC values varying from 0.21 to 0.30, 0.31 to 0.40 and 0.41 to 0.50 contained about 5.3, 7.9 and 15.5 % of the total markers. The *P* value ranged from 42.2 to 96.7 % with an average of 79.3 %. Reproducibility percentage and call rate were also very high, varying from 96.8 to 100 % and 80.5 to 100 %, respectively whereas, discordance values were $\leq 0.01$ for DArT markers.

### 4.4.5 Evaluation of population structure and relationships among chickpea genotypes

The highest value for the probability of data likelihood [LnP(D)] was obtained at $K = 8$, but with very high standard deviation (Figure 4.3a). However, maximum $\Delta K$ value was observed at $K = 2$ (Figure 4.3b), suggesting that the germplasm collection was mainly composed of two sub-populations (Figure 4.3c). Chickpea genotypes having less than 80 % inferred ancestry (obtained from Q-matrix using fastSTRUCTURE version 1.0), were considered as admixtures. Chickpea germplasm collection contained a total of 21 admixtures. Consequently, sub-population 1 (Pop 1) had 57 chickpea genotypes including 30 desi and 27 kabuli types whereas, 90 genotypes (69 desi and 21 kabuli types) were grouped as sub-population 2 (Pop 2). Marker data of 147 chickpea genotypes (excluding admixtures) were used to analyze molecular variance that showed highly significant ($P \leq 0.001$) genetic variation within and between two sub-populations (Pop 1 and 2). The variation within sub-populations contributed 64 % to the total variation present in the germplasm collection whereas, the remaining 36 % was contributed by variation between two sub-populations (Table 4.6). Analysis of principal coordinates was performed to show the relationship among different

**Table 4.4** Correlations among selected seed constituent traits.

| Botanical type | Genotype | Seed trait | Starch | Amylose | Protein |
|---|---|---|---|---|---|
| Desi | 121 | TSW | 0.67** | -0.30** | -0.57** |
| | | Starch | | -0.26** | -0.54** |
| | | Amylose | | | 0.31** |
| Kabuli | 56 | TSW | 0.68** | -0.04ns | -0.30* |
| | | Starch | | -0.03ns | -0.36** |
| | | Amylose | | | 0.10ns |
| Overall | 177 | TSW | 0.54** | -0.08ns | -0.13** |
| | | Starch | | -0.27** | -0.40** |
| | | Amylose | | | 0.48** |

** and * significant at the $P \leq 0.01$ and 0.05, respectively. 'ns' stands for 'not significant'.

**Table 4.5** Shannon diversity index estimates for the composite collection.

| Geographical region | SDI | | | |
|---|---|---|---|---|
| | TSW | Starch | Protein | Amylose |
| Europe | 0.58 | 0.51 | 0.62 | 0.61 |
| North Africa | 0.65 | 0.60 | 0.47 | 0.49 |
| South Asia | 0.55 | 0.52 | 0.43 | 0.45 |
| Southwest Asia | 0.59 | 0.60 | 0.56 | 0.58 |
| Sub-Saharan Africa | 0.53 | 0.65 | 0.63 | 0.59 |

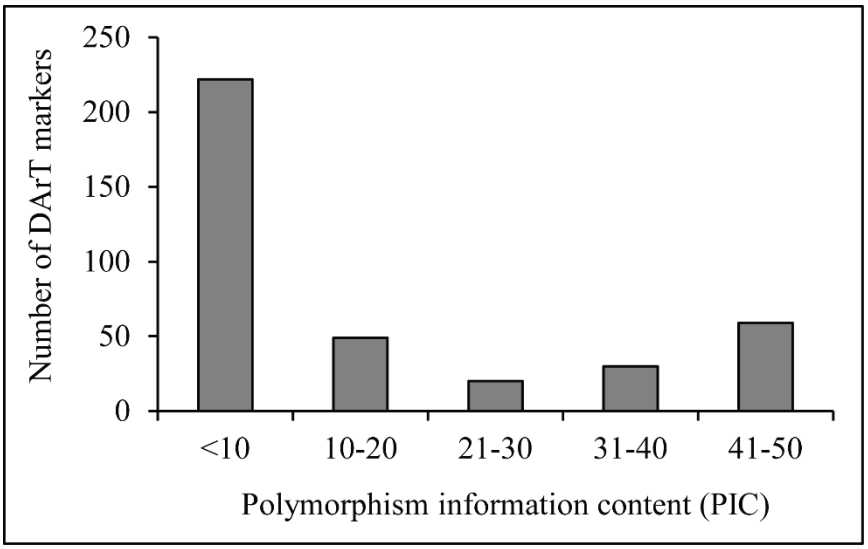


**Figure 4.2** Frequency distribution of polymorphism information content (PIC) values of 380 DArT markers in 168 chickpea genotypes.

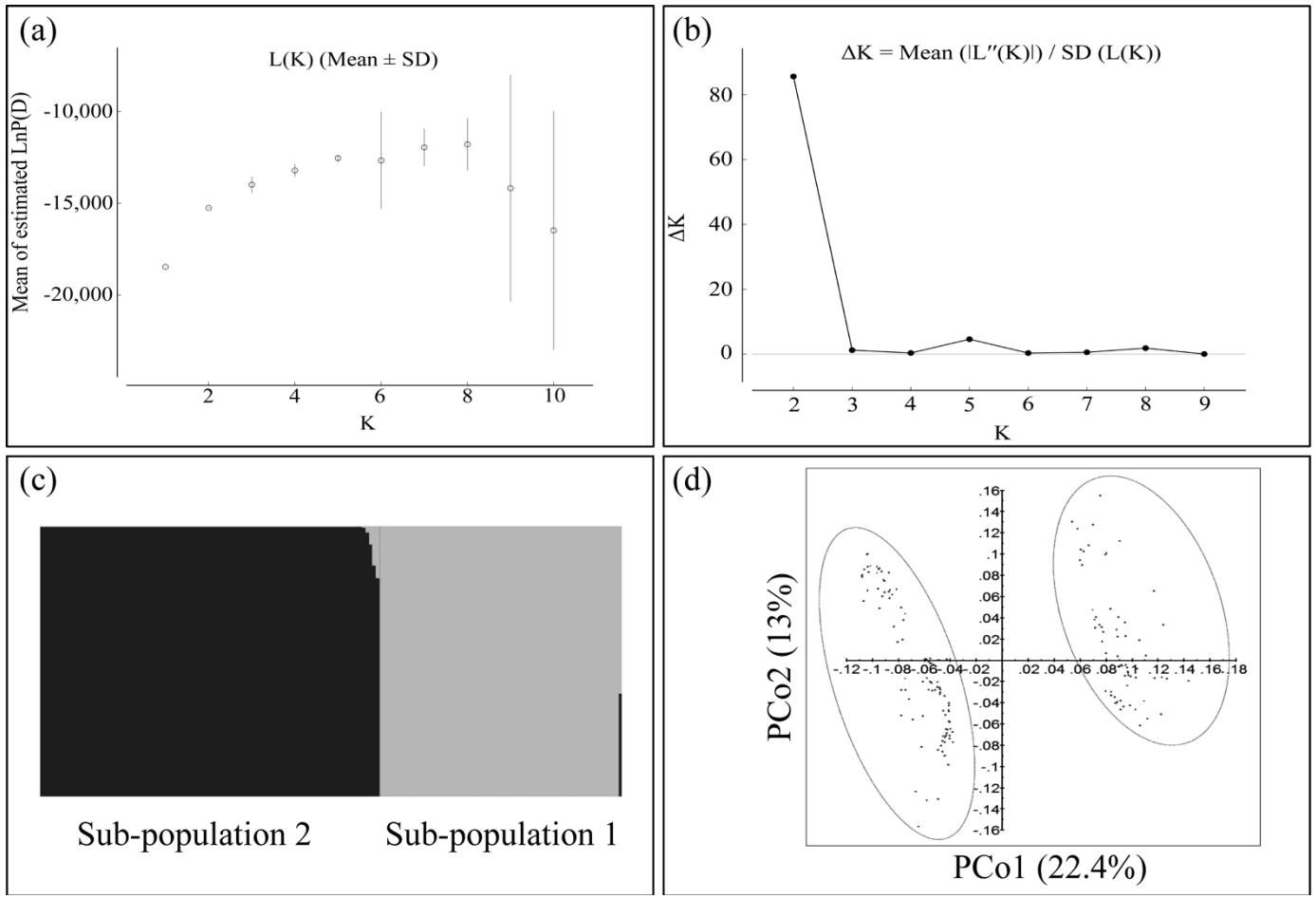


**Figure 4.3** Population structure assessment for germplasm collection of 168 chickpea genotypes based on 380 DArT markers.

**(a)** estimated mean probability of data likelihood [LnP(D)], **(b)** *ΔK* values for a given *K*, **(c)** genetic composition of the germplasm collection assessed in STRUCTURE program, and **(d)** Principal coordinate (PCo) analysis of chickpea genotypes where first two coordinates explained 22.4 and 13% of total variation. The LnP(D) estimates at each *K* value were determined by STRUCTURE version 2.3.4 and results were analyzed using Structure Harvester version 0.6.94. SD stands for standard deviation.

**Table 4.6** Analysis of molecular variance (AMOVA) for variation distribution between and within sub-populations.

| Source | DF | MS | Est. Var. | % |
|---|---|---|---|---|
| Between Pops | 1 | 1022.6 | 14.3 | 36% |
| Within Pops | 145 | 25.9 | 25.9 | 64% |
| Total | 146 | | 40.2 | 100% |

Data is significant at $P \leq 0.001$.

chickpea genotypes. Among the top five components, the first (PCo1) and second (PCo2) principal coordinates accounted for 22.4 and 13.0 % of the total variation present in germplasm collection, respectively (Figure 4.3d).

Phylogenetic analysis categorized 168 chickpea genotypes into two distinct clusters and each with four sub-clusters (Figure 4.4). Cluster I was composed of 70 chickpea genotypes (42 desi and 28 kabuli genotypes) whereas, cluster II had 98 chickpea genotypes (73 desi and 25 kabuli genotypes). Desi and kabuli chickpea genotypes were separated into different sub-clusters. Sub-cluster Ia predominantly contained kabuli genotypes, *i.e.* 22 out of 23 genotypes (95.7 %) were of kabuli type. However, 91.3, 91.7, 87.5 and 90.5 % genotypes were of desi types in sub-clusters Ic, Id, IIa and IIc, respectively. In sub-clusters Ib and IId, about 72.7 and 71.4 % of genotypes were of desi types, respectively. Sub-cluster IIb contained approximately equal number of desi (52.8 %) and kabuli (47.2 %) chickpea genotypes. Chickpea genotypes could not be separated on the basis of their geographical origins.

### 4.4.6 Marker-trait association (MTA) analysis

Mixed linear model (MLM) approach identified a total of 33 out of 380 DArT markers to be highly associated (significant at $P \leq 0.05$) with the selected seed constituent traits (TSW, and concentrations of total starch, protein and amylose) in the chickpea composite collection. In desi genotypes, 19 markers were associated with TSW, total starch, protein and amylose (Table 4.7). Two markers, cpPb-677692 and cpPb-172207, were significantly ($P \leq 0.05$) associated with TSW in desi genotypes explaining 5.1 and 4.2 % of the total variation, respectively. Protein, total starch and amylose in desi genotypes were significantly ($P \leq 0.05$) associated with six (cpPb-677712, cpPb-491461, cpPb-678060, cpPb-681104, cpPb-677692 and cpPb-676245), eight (cpPb-327841, cpPb-680741, cpPb-322806, cpPb-677249, cpPb-172194, cpPb-677692, cpPb-488627 and cpPb-489318) and three (cpPb-676079, cpPb-682003 and cpPb-327841) DArT markers that explained 6.0-7.9, 3.7-4.8 and 4.1-5.8% of the total variation, respectively.

In kabuli genotypes, 14 DArT markers showed a significant association with selected seed constituent traits (Table 4.7). TSW, protein and total starch were significantly ($P \leq 0.05$) associated with four (cpPb-680201, cpPb-680572, cpPb-489549 and cpPb-677783), six (cpPb-171485, cpPb-677798, cpPb-490962, cpPb-677056, cpPb-677672 and cpPb-173447) and three (cpPb-490970, cpPb-676245 and cpPb-677783) DArT markers explaining 6.9-10.3, 8.4-9.0 and 6.0-16.1 % of total variation, respectively. However, only one significantly ($P \leq 0.05$) associated DArT marker, cpPb-677529, was identified for amylose concentration in kabuli

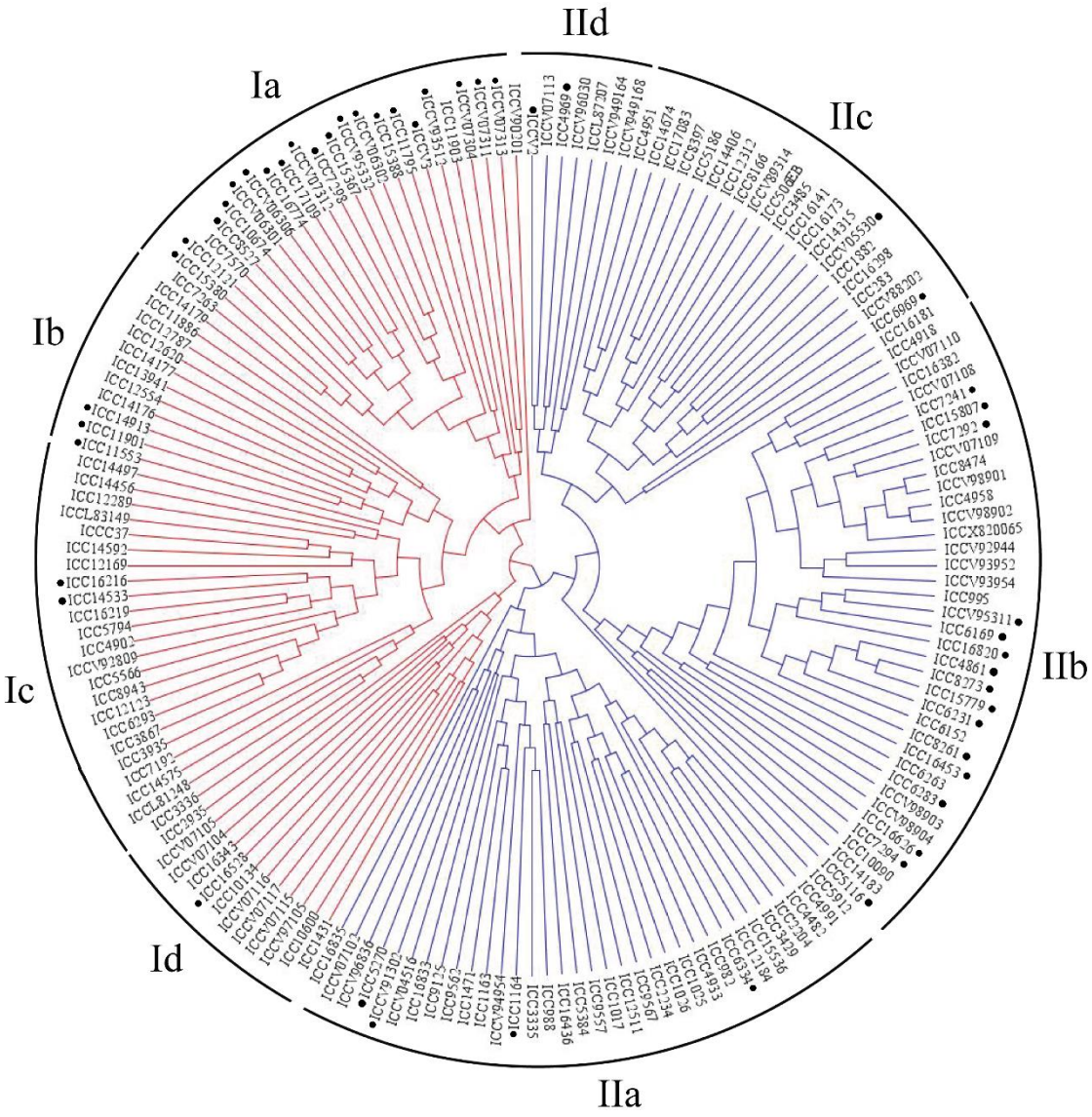


**Figure 4.4** Phylogenetic relationships among 168 chickpea genotypes used in the study showing two main clusters (red and blue lines) divided into four sub-clusters each.

Genotypes with dark spots represent kabuli type, whereas the rest are desi type.

**Table 4.7** DArT markers significantly associated with selected seed constituent traits in desi and kabuli chickpea genotypes using mixed linear model.

| Trait | Marker | $F_{marker}$ | $P$ | $R^2$ |
|---|---|---|---|---|
| | **Desi genotypes** | | | |
| TSW | cpPb-677692 | 6.008 | 0.016 | 0.051 |
| | cpPb-172207 | 5.225 | 0.024 | 0.042 |
| Protein | cpPb-677712 | 9.050 | 0.003 | 0.079 |
| | cpPb-491461 | 9.050 | 0.003 | 0.079 |
| | cpPb-678060 | 9.050 | 0.003 | 0.079 |
| | cpPb-681104 | 9.050 | 0.003 | 0.079 |
| | cpPb-677692 | 6.129 | 0.015 | 0.060 |
| | cpPb-676245 | 5.739 | 0.018 | 0.051 |
| Total starch | cpPb-327841 | 5.575 | 0.020 | 0.048 |
| | cpPb-680741 | 5.443 | 0.021 | 0.048 |
| | cpPb-322806 | 5.185 | 0.025 | 0.044 |
| | cpPb-677249 | 5.092 | 0.026 | 0.043 |
| | cpPb-172194 | 4.742 | 0.032 | 0.040 |
| | cpPb-677692 | 4.484 | 0.037 | 0.040 |
| | cpPb-488627 | 4.381 | 0.039 | 0.037 |
| | cpPb-489318 | 4.371 | 0.039 | 0.037 |
| Amylose | cpPb-676079 | 6.243 | 0.014 | 0.058 |
| | cpPb-682003 | 5.387 | 0.022 | 0.047 |
| | cpPb-327841 | 4.702 | 0.032 | 0.041 |
| | **Kabuli genotypes** | | | |
| TSW | cpPb-680201 | 7.201 | 0.010 | 0.103 |
| | cpPb-680572 | 6.260 | 0.016 | 0.090 |
| | cpPb-489549 | 5.037 | 0.029 | 0.076 |
| | cpPb-677783 | 4.846 | 0.032 | 0.069 |
| Protein | cpPb-171485 | 4.446 | 0.040 | 0.084 |
| | cpPb-677798 | 4.446 | 0.040 | 0.084 |
| | cpPb-490962 | 4.446 | 0.040 | 0.084 |
| | cpPb-677056 | 4.446 | 0.040 | 0.084 |
| | cpPb-677672 | 4.446 | 0.040 | 0.084 |
| | cpPb-173447 | 4.406 | 0.041 | 0.090 |
| Total starch | cpPb-490970 | 11.171 | 0.002 | 0.161 |
| | cpPb-676245 | 5.391 | 0.024 | 0.075 |
| | cpPb-677783 | 4.356 | 0.042 | 0.060 |
| Amylose | cpPb-677529 | 5.549 | 0.023 | 0.110 |

genotypes which explained 11 % of the total variation.

### 4.5 Discussion

To improve seed constituent traits in chickpea, identification of important plant genetic resources (Cardi, 2016) and strong trait-associated molecular markers (Collard and Mackill, 2008) are essential to initiate and accelerate chickpea improvement programs. Very limited studies are available that report genetic resources and associated markers for chickpea seed constituent traits (Jadhav et al., 2015). Therefore, a genome-wide association study (GWAS) using DArT markers was performed for selected seed constituent traits in a diverse collection of chickpea genotypes.

In the present study, selected seed constituent traits (TSW, total starch, protein and amylose) varied significantly among 168 chickpea genotypes grown in three environments (Figure 4.1) indicating that the chickpea germplasm collection is useful for GWAS. The higher TSW (g) and total starch concentration (%) in kabuli genotypes (229.2 ± 46.8 g and 42.9 ± 1.9 %) compared to desi types (198.0 ± 19.6 g and 38.0 ± 1.8 %) concurred with the conclusion of Kujur et al. (2014) and Frimpong et al. (2009), respectively. However, average values for protein (20.8 ± 2.6 and 21.3 ± 3.3 %) and amylose (32.4 ± 1.7 and 32.2 ± 1.7 % of total starch) concentrations did not show significant difference between desi and kabuli genotypes, which is in agreement with the results of Frimpong et al. (2009). The selected seed constituent traits in chickpea were significantly influenced by G, E and G×E. The medium to high $H^2$ of seed constituent traits (total starch, protein and amylose) and high $H^2$ of extrinsic trait TSW, suggests the higher environmental sensitivity of the former (Table 4.3). Biosynthesis of starch, amylose and protein is complex in plants and includes a number of genes/enzymes affected by environment and thus affect the accumulation of the final product (Dupont and Altenbach, 2003; Thitisaksakul et al., 2012).

Starch and protein are the main constituents of chickpea seeds whereas, amylose together with amylopectin constitute a starch granule. Starch and protein biosynthesis depend on carbon to nitrogen ratio (C:N) and have shown positive and negative correlations with C:N, respectively (Quyen et al., 2013). Therefore, a negative correlation between starch and protein concentrations was observed in the present study. The positive correlation between starch concentration and seed weight is similar to that reported in lentil (Tahir et al., 2011). Starch biosynthesis favors amylopectin accumulation as it is essential for the granular structure (Zeeman et al., 2010) thus showed a negative correlation with amylose in the present study. Being correlated to starch negatively, protein and amylose showed a positive correlation in

chickpea genotypes. Shannon-Weaver diversity index (SDI) was calculated to show the representation of each region in the germplasm collection. SDI for Meso-, North- and South-America was not calculated as they included only a limited number of genotypes compared to other regions. SDI values showed no association with number of genotypes or centers of origin/distribution.

The natural variation for selected seed constituent traits in the chickpea composite collection was utilized to associate phenotypic variation with DArT markers that have been applied to whole genome profiling of several staple crops since the introduction of the DNA array technology (Jaccoud et al., 2001). DArT markers are bi-allelic dominant markers and show low polymorphism as a result of having been developed from genomic segments with low copy number (10 % of the whole genome). However, DArT markers have better reproducibility, through-put and cost-effective features compared to RAPD, AFLP and other conventional PCR based markers (Wenzl et al., 2004; Heller-Uszynska et al., 2011; Howard et al., 2011). Therefore, more than 25 studies published during 2014 have implemented DArT markers to explore genetic diversity for various traits in different crops (http://www.diversityarrays.com/dart-resources-papers).

In the present study, 380 non-redundant polymorphic DArT markers were identified, out of which approximately 23.4 % markers showed PIC values > 0.30. Thudi et al. (2011) also reported a low average PIC value of 0.13 for DArT markers in 94 genotypes of nine different *Cicer* species (*C. arietinum, C. bijugum, C. cuneatum, C. echinospermum, C. judaicum, C. Microphyllum, C. pinnatifidum, C. Reticulatum* and *C. yamashitae*), with only 11.7 % of total markers having PIC values > 0.30 whereas, 81.7 % markers showed PIC values < 0.20. The narrow genetic background of chickpea (Abbo et al., 2003) might be the reason for low PIC values in the present and previous study (Thudi et al., 2011).

Population structure of the germplasm collection was determined by STRUCTURE using $\Delta K$ method and was confirmed by analyzing phylogenetic relationships and PCoA. Consequently, two distinct sub-populations were identified irrespective of botanical types and geographical origins. However, phylogenetic analysis distinguished some desi and kabuli genotypes into separate sub-clusters. The random grouping of chickpea genotypes might be due to different ancestry of genotypes, random events of evolutionary forces such as migration, mutation, selection, genetic drift or germplasm exchange by human intervention among adjacent areas. These forces could have separated chickpea genotypes into different gene pools (Keneni et al., 2012).

Association study evaluates co-variation between traits and associated genes, and

measures to what extent they vary together in a population using the underlying orderly co-segregation between different loci (Zondervan and Cardon, 2004). The present report, to best of our knowledge in chickpea, is the first DArT marker based association mapping study for selected seed constituent traits such as protein, total starch and amylose. TSW, a major agronomic trait, has been studied extensively in previous reports (Kujur et al., 2014; Thudi et al., 2014; Bajaj et al., 2015; Kujur et al., 2015a; Kujur et al., 2015b). A marker-trait association study was recently reported for protein content in 187 chickpea genotypes but with only 23 simple sequence repeats markers (Jadhav et al., 2015). To discover genome-wide marker-trait associations in desi and kabuli genotypes, MLM was used as it takes into account kinship among genotypes for association analysis in contrast to general linear model (Zhang et al., 2010b). The significant trait-associated DArT markers explained, on an average, higher phenotypic variation for kabuli genotypes (6.0 – 16.1 %) compared to desi types (3.7 – 7.9 %). TSW associated DArT markers explained 4.2 – 10.3 % of total phenotypic variation which is low compared to 8.7 – 36.9 % reported by Thudi et al. (2014). However, Thudi et al. (2014) included seven wild *Cicer* species in their reference set that might have contributed to a higher degree of phenotypic variation and therefore, associated markers explained a high phenotypic variation. The phenotypic variation explained by protein associated markers in the present study was 5.1 – 9.0 % which is higher than that of 2.4 – 5.1 % reported by Jadhav et al. (2015). Trait-associated DArT markers and selected genotypes can be validated and deployed to improve above mentioned traits through molecular breeding in chickpea.

# CHAPTER 5. GENETIC ANALYSIS THROUGH GENOTYPING BY SEQUENCING DISCOVERS NOVEL QUANTITATIVE TRAIT LOCI FOR SELECTED SEED CONSTITUENT TRAITS IN CHICKPEA (*Cicer arietinum* L.)

## 5.1 Study 3*

The third study utilized a recombinant inbred line (RIL) population developed using two parents with contrasting protein concentrations. Genotyping by sequencing was used to identify single nucleotide polymorphisms (SNP) to develop linkage maps and putative candidate genes for chickpea seed quality improvement.

* Wang, R., Gangola, M. P., Jaiswal, S., Irivine, C., Gaur, P. M., Båga, M., and Chibbar, R. N. 2016. Genetic analysis through genotyping by sequencing discovers novel quantitative trait loci for selected seed constituent traits in chickpea (*Cicer arietinum* L.) (to be submitted).

## 5.2 Introduction

Chickpea (*Cicer arietinum* L.) is an annual diploid (2n = 2x = 16) domesticated leguminous crop species. It ranked second only to dry beans for total annual pulse-crop production during 2014 (FAOSTAT, 2014). Chickpea seeds are an excellent source of nutrients promoting human and animal health (Wood and Grusak, 2007; Yadav et al., 2007). Chickpea seeds contain starch (40 – 60 %), protein (17 – 30 %), and lipids (6 – 9 %) as major seed constituents (Wood and Grusak, 2007). Chickpea protein has a higher concentration of an essential amino acid lysine (4.9-7.7 g/100 g protein) compared to cereal grains (~2.8 g/100 g protein). However, sulphur-containing amino acids methionine and cysteine are deficient in chickpea compared to cereals. Therefore, consumption of pulses with cereals in 2 – 4:1 provides a balanced proportion of amino acids (Wood and Grusak, 2007). High concentration of amylose (30 – 40 % of total starch) and dietary fibers (18 – 22 %) in chickpea seeds slows the release of sugars into the blood thus limits the risk and occurrence of diabetes (Chibbar et al., 2010; Jukanti et al., 2012). Polyunsaturated-, monounsaturated- and saturated- fatty acids account for 66, 19 and 15 %, respectively, of the total fat content in chickpea seeds (Jukanti et al., 2012). Chickpea has a good complement of vitamins; the predominant being tocopherol and β-carotene. Chickpea seeds are also rich in minerals including K, P, Ca, Fe, Cu, Zn, Mn and Mg and can meet daily dietary requirement (Wang et al., 2010a; Jukanti et al., 2012; Khan et al., 2015). Therefore, chickpea seeds are an important component of vegetarian diet mainly in developing countries.

The advancement in genomic technologies has assisted in the dissection of genetic control of valuable agronomic traits in chickpea (Gaur et al., 2012) such as leaf characters, growth habit, pigmentation and flowering time (Banerjee et al., 2001; Cho et al., 2002; Cobos et al., 2007; Ali et al., 2015; Das et al., 2015). Quantitative trait loci (QTLs) associated with abiotic and biotic strtesses in chickpea have also been explored widely including tolerance/resistance to Ascochyta blight (Cobos et al., 2006; Iruela et al., 2006; Tar'an et al., 2007), Botrytis grey mold (Anuradha et al., 2011), Fusarium wilt, salt and drought stress (Sabbavarapu et al., 2013; Patil et al., 2014). A few studies also define QTLs associated with extrinsic grain quality contributors, *viz.* yield, seed weight, seed size, seed coat color and seed coat thickness (Cobos et al., 2007; Cobos et al., 2009; Vadez et al., 2012; Kujur et al., 2015a; Kujur et al., 2015b). However, similar genetic studies for intrinsic seed constituent traits, such as seed storage compounds, are very limited in chickpea. To assist plant breeders in developing novel breeding strategies to improve chickpea seed quality and the value of its utilization, efforts must be made on the genetic analysis for important seed storage compounds.

The first linkage analysis for chickpea was conducted with isozyme markers (Gaur and Slinkard, 1990a). Since then, a wide range of PCR-based markers have been used for QTL studies in chickpea (Gaur et al., 2012). As next-generation sequencing (NGS) progresses, genotyping by sequencing (GBS) is becoming popular as a high-throughput genotyping approach (Elshire et al., 2011) to improve the resolution and power of QTL mapping. GBS assisted QTL studies in chickpea have been successfully used to develop high density linkage maps and identification of QTL associated with agronomic traits (Kujur et al., 2015b; Verma et al., 2015). The present study utilized GBS based whole genome SNP discovery technique to investigate the genetic control of selected seed constituent traits in chickpea to identify QTLs associated with selected seed constituent traits such as 1000-seed weight (TSW) and concentrations of total starch, protein and amylose.

## 5.3 Material and methods

### 5.3.1 Plant material

A mapping population, consisting of 222 recombinant inbred lines (RIL), was derived from a cross between two desi chickpea types ICC 995 (Indian origin) × ICC 5912 (Mexican origin) at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, Patancheru, India). The population was grown at four different locations: (i) ICRISAT (17 ° 53 ′ N latitude, 78 ° 27 ′ E longitude and 545 m altitude, Patancheru, India) during 2011-2012 (F 2011), (ii) Biggar (52 ° 3 ' N latitude, 107 ° 59 ' W longitude and 649 m altitude, SK, Canada) in 2012 (F 2012), (iii) agricultural greenhouse (52 ° 07 ′ N latitude, 106 ° 38 ′ W longitude and 481.5 m altitude, University of Saskatchewan, Saskatoon, SK, Canada) in 2012 (G 2012), and (iv) Aberdeen (52 ° 34 ′ N latitude, 106 ° 29 ′ W longitude and 517 m altitude, SK, Canada) in 2013 (F 2013). Field experiments were performed in a randomized complete block design during October to mid-March at ICRISAT and June to mid-September at all other locations. Fifty seeds of each line were sown with 15 and 25 cm spacing between plants and rows, respectively. Fertilizers (22 kg/ha $P_2O_5$ and 40 kg/ha N) were applied before seeding. The mean minimum and maximum temperatures were 15.1 °C and 31.9 °C with an average precipitation of 2.3 mm during 2011 – 2012 at ICRISAT. At Biggar, mean minimum and maximum temperatures were 7.8 °C and 14.4 °C with an average precipitation of 1.8 mm during 2012. The mean minimum and maximum temperatures were 9.9 °C and 16.7 °C, with a mean precipitation of 1.4 mm during the growing season at Aberdeen in 2013. In the greenhouse, average daily minimum and maximum temperatures were 18 °C and 23 °C with an 18 h photoperiod. Plants were harvested and seeds were stored at room temperature. All genotypes

were grown in two blocks. In each block, 50 seeds of one genotype were sown in a single row. When harvested, all seeds from the 50 individual plants of the same genotype were pooled. For each genotype, the selected seed constituent traits were analyzed two times for each block, giving two biological replicates for each block. For each biological replicate in each block, two technical replicates were analyzed, giving eight replicates in total. Field trial of 2011 was carried out by researchers and employees at ICRISAT following similar growing method. Chickpea seed of the field trial in 2011 was imported for analysis.

### 5.3.2 Seed weight analysis

One hundred seeds were counted using an electronic seed counter (Seedburo Equipment Co., Chicago, IL, USA) and weighed. The weight was multiplied by ten to obtain one thousand seed weight (TSW).

### 5.3.3 Grinding of seed material

Chickpea seeds (about 10 g) were ground into a fine meal using a UDY cyclone mill (Udy Corporation, Fort Collins, CO, USA) to pass through a 0.5 mm sieve. The seed meal was collected, stored at room temperature and used to determine total starch, amylose and protein concentrations.

### 5.3.4 Determination of total starch concentration

Total starch concentration in chickpea seed meal (100 ± 0.5 mg) was determined by a enzymatic hydrolysis method using a commercial kit (Megazyme International Ireland Ltd., Wicklow, Ireland) (McCleary et al., 1997).

### 5.3.5 Amylose determination

Starch was purified from chickpea seed meal following a modified method (Peng et al., 1999) involving cesium chloride (CsCl) density gradient centrifugation (Asare et al., 2011). The purified starch was used to determine amylose concentration using iodine based method with some modifications (Mahmooda et al., 2007); therefore, it is expressed as percentage of total starch. In brief, purified starch (5 mg) was weighed in a 2 mL centrifuge tube. The starch was sequentially suspended in 95 % (v/v) ethanol (75 µL), 1M NaOH (450 µL) and distilled water with proper shaking before adding next solution. The mixture was mixed well and incubated at room temperature for 1 h. Thereafter, an aliquot (200 µL) was taken out in a 15 mL disposable tube and neutralized with 0.05M citric acid (1 mL), followed by addition of 800

µL of $I_2$/KI solution [0.8 g iodine ($I_2$) and 8 g potassium iodide (KI) in 1 L of distilled water]. The mixture was mixed well and volume was made up to 12 mL with distilled water. The absorbance was observed at 535 and 620 nm for amylopectin and amylose, respectively.

### 5.3.6 Estimation of protein concentration

Total nitrogen was determined by combustion method (FP-528 Protein/Nitrogen Analyser, Leco Corporation, St Joseph, MI, USA) (Frimpong et al., 2009). Protein concentration was calculated by using following formulae (Karaca et al., 2011):

protein (%) = % N × nitrogen to protein conversion factor (6.25 for chickpea seeds)……(5.1).

### 5.3.7 Statistical analysis

Statistical analysis including box-plots, analysis of variance (using mixed model) and Pearson's correlation coefficients, was performed using MINITAB 14 statistical software (Minitab Inc., State College, PA, USA). Phenotypic classes were prepared by using MINITAB 14 statistical software (Minitab Inc., State College, PA, USA). Mixed model was used to calculate analysis of variance (ANOVA) in MINITAB 14 and resulted F values from ANOVA was utilized to determine the significance of the influences from genotype (G), environment (E) and their interaction (G×E). Covariance estimates of variance components were used to calculate heritability ($H^2$) as described by Singh et al. (1993).

### 5.3.8 DNA extraction and genotyping by sequencing

Three seeds from each genotype of the mapping population were grown in the greenhouse of the University of Saskatchewan. Young leaves (a pool of three leaves from different plants) were collected two weeks after seeding. The leaves were immediately frozen with liquid nitrogen and stored at -80°C. Genomic DNA was isolated following the manufacturer's protocol (Qiagen DNeasy Plant Mini Kit, Qiagen, Germantown, MD). Genomic DNA was quantified using Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, Molecular Probes, Eugene, OR, USA) and normalized to 10 ng/µL. Libraries for Ion Proton genotyping by sequencing (GBS) were prepared as described by Mascher et al. (2013) at the Plateforme d'analyses génomiques of the Institut de Biologie Intégrative et des Systèmes (IBIS, Université Laval, Québec, Canada) with the following exceptions: *ApeKI* with corresponding barcodes were used instead of the *PstI*/*MspI* combination and a blue Pippin (SAGE Science Inc., Beverly, MA, USA) was used to size libraries before PCR amplification. Libraries were prepared for sequencing using an Ion CHEF, Hi-Q reagents and P1 V3 chips (ThermoFisher

Scientific, Life Technologies Inc., ON, Canada) and the sequencing was performed on a Ion Proton sequencer (ThermoFisher Scientific, Life Technologies Inc., ON, Canada) for 300 flows also at IBIS (Université Laval, Québec, Canada).

### 5.3.9 Genetic mapping

Sequencing data obtained from the GBS assay were processed to construct a genetic map of the intra-specific population of ICC 995 × ICC 5912. The raw 'fastq' files, obtained from Université Laval, were processed through Trimmomatic version 0.32 (Bolger et al., 2014) to remove adaptors/ barcodes/reads less than 36 bp, perform sliding window quality filter and crop remaining reads that are more than 170 bp down to 170 bp. Output sequences from Trimmomatic were aligned to the reference genome of a desi type chickpea (http://cicer.info/databases.php) using Bowtie 2.0 (Langmead and Salzberg, 2012) and SNP variables were called by Sequence Alignment/Map tools version 0.1.19 (SAMtools; Li, 2011). The whole genome SNP variable calls detected by SAMtools were filtered using Variant Call Format tools version 0.1.12 (VCFtools; Danecek et al., 2011). The following filters were applied to the data: minimum allele frequency of 0.05, maximum missing data of 0.8, minimum depth of 6 and thinning of 100. SNP markers were excluded when they: (i) had more than 20% missing information, (ii) were heterozygous in parental lines, and (iii) had the same variable calls in both parental lines. In-dels were also removed during filtering process. The filtered SNP markers were clustered into linkage groups using the LOD ≥ 5.0 and were ordered with the algorithm of RECORD and Kosambi mapping function using QTL Icimapping v4.1 (Meng et al., 2015). Fine tuning of marker order was performed using the rippling method of COUNT with the window of 5.0. The genetic map with shortest distance was preferred. Linkage map construction was carried out in MapChart v2.3 (Voorrips, 2002).

For QTL identification, the genotypic data of the mapped SNPs on the intra-specific linkage map was integrated with the field phenotypic data of 1000 seed weight (TSW), total starch, amylose content and protein concentration. QTL analysis was done using QTL Icimapping version 4.1 based on inclusive composite interval mapping (ICIM) (Li et al., 2015). The LOD threshold was determined using a 1000 permutation test. The software also estimated the epistatic effect for each QTL identified for each trait. QTL Icimapping illustrated the identified QTLs diagrammatically. QTL analysis was also performed with software package MapQTL version 5 using similar parameters as in Icimapping v4.1.

### 5.3.10 Identification of genes in robust regions of QTL

To identify putative candidate genes associated with TSW and selected seed constituent traits, QTLs with flanking markers on the same chromosome and explaining higher phenotypic variance were analyzed. Physical positions of the SNPs were utilized to identify the genomic region on the reference genome of desi chickpea (http://cicer.info/cgi-bin/gb2/gbrowse/desi/). Genes with UniRef90 annotations were downloaded and analyzed for gene ontology using online tools (http://www.uniprot.org/ and http://www.ebi.ac.uk/QuickGO-Beta/).

## 5.4 Results

### 5.4.1 Variation for seed quality straits in RILs

RILs, grown in different environments, were characterized for thousand seed weight (TSW), total starch, protein and amylose concentrations (Figure 5.1). Parent ICC 995 had higher seed weight and starch concentration but lower amylose and protein concentrations compared to the other parent, ICC 5912. Coefficient of variation (CV) was the highest for seed weight (0.20 to 0.39) followed by total starch (0.10 to 0.17), protein (0.10 to 0.14) and amylose (0.03 to 0.09) concentrations in each growing location (Appendix 6). RIL showed transgressive segregation for all the seed constituent traits (Appendix 6). Seed weight was the highest for F 2013 (208.7 ± 42.7 g) followed by G 2012 (179.8 ± 69.3 g), F 2011 (175.5 ± 38.9 g) and F 2012 (101.1 ± 30.2 g), respectively. Protein and amylose concentrations were also the highest in genotypes grown in F 2013 ranging from 16.0 to 26.8 % and 33.1 to 39.5 % (of total starch), respectively. However, maximum accumulation of total starch was observed in F 2011 grown genotypes ranging from 31.8 to 52.0 % with an average of 42.2 ± 3.9 g. RILs with consistently higher seed weight and total starch concentration were ICCRIL07-0184, ICCRIL07-0149, ICCRIL07-0201, ICCRIL07-0170 and ICCRIL07-0002; whereas, ICCRIL07-0232 and ICCRIL07-0198 (Appendix 7) were identified for consistently higher amylose and protein concentrations across all the environments.

### 5.4.2 Correlation among seed traits in RILs

Seed weight showed a significant positive correlation to total starch ($r = 0.55$; $P < 0.01$) concentration but negative to amylose ($r = -0.20$; $P < 0.01$) and protein concentrations ($r = -0.78$; $P < 0.01$). Accordingly, total starch concentration was also negatively correlated to amylose ($r = -0.33$; $P < 0.01$) and protein concentrations ($r = -0.55$; $P < 0.01$). Amylose concentration had significant positive correlation ($r = 0.19$; $P < 0.01$) with protein concentration in chickpea seeds (Figure. 5.2).

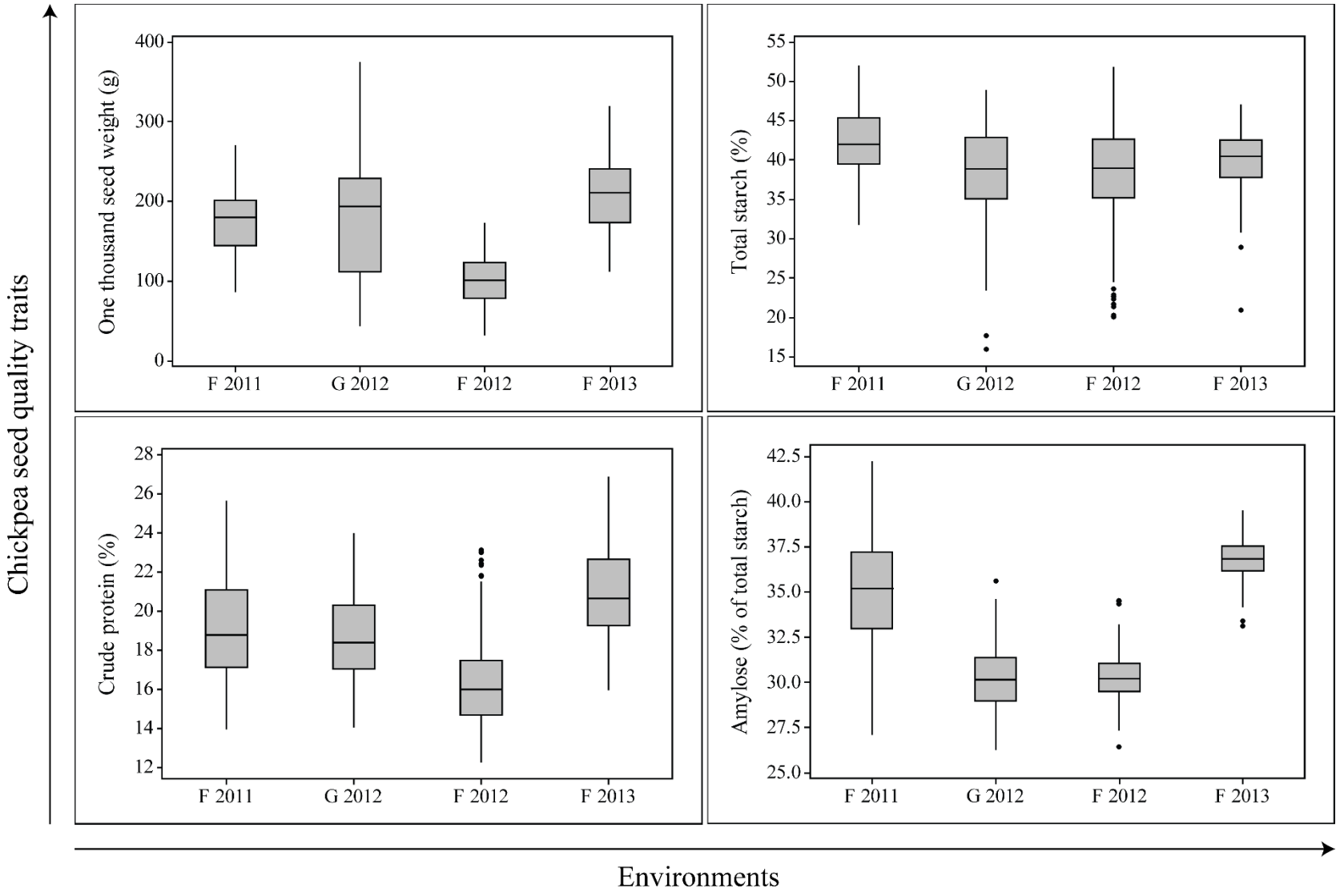


**Figure 5.1** Boxplot analysis of recombinant inbred lines (RILs) for selected seed constituent traits.
RILs were grown in four environments at ICRISAT (F 2011), Biggar (F 2012), agricultural greenhouse (G 2012) and Aberdeen (F 2013). The upper and lower error bars represent the non-outlier range of the data set. The box represents the interquartile range (IQR), whereas the middle line shows the median value of the data set. The dark circles represent the outliers, calculated as the data points out of the 1.5 times the IQR.

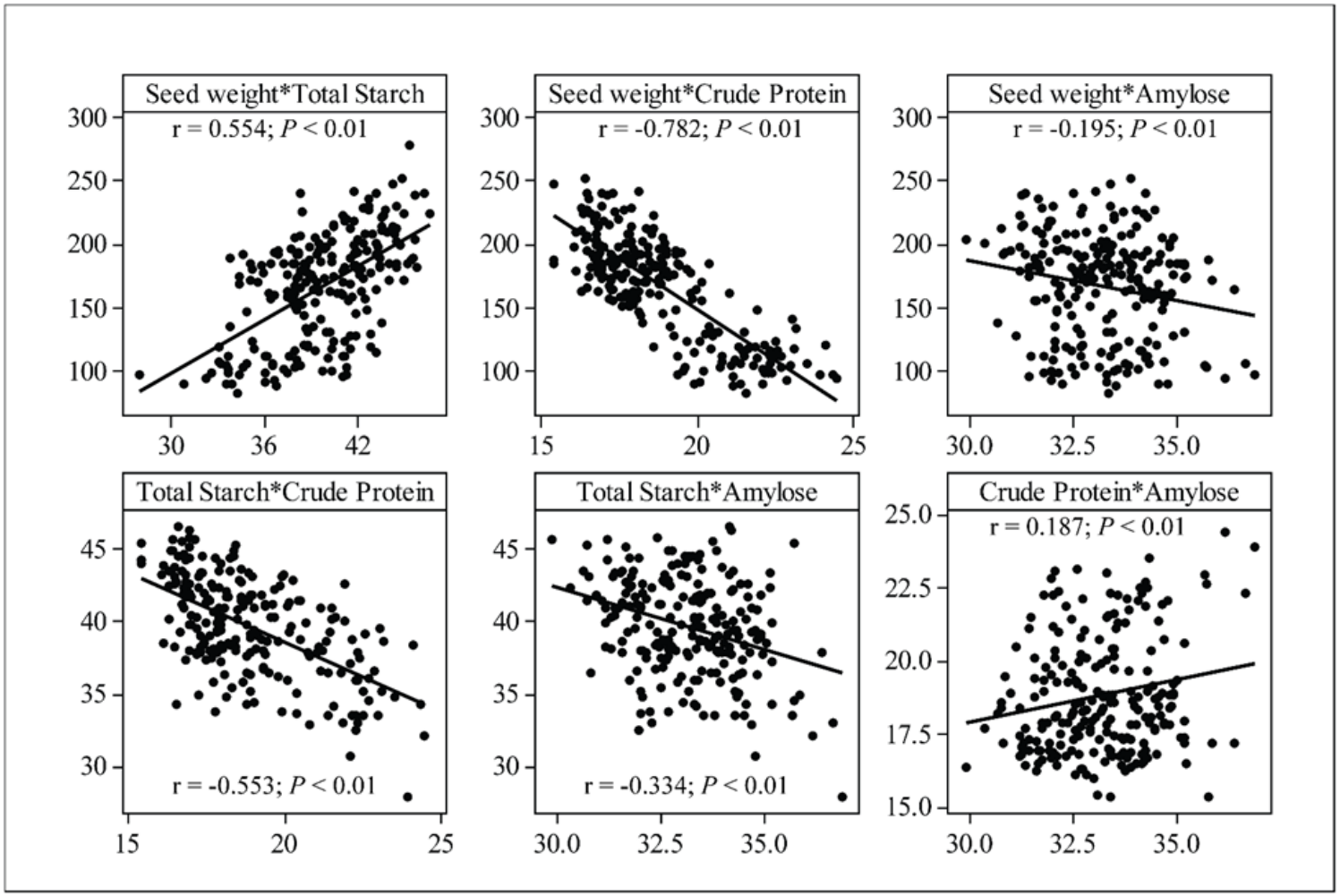


**Figure 5.2** Scatter-plot analysis of recombinant inbred lines representing correlation among selected seed constituent traits in chickpea.
The regression line represents the correlation (r = Pearson's correlation coefficient and *P* = probability level) between two traits.

### 5.4.3 Effect of genotype and environment on seed constituent traits and their broad sense heritability

Analysis of variance (ANOVA) of data from the multi-environment experiment with RILs established a significant ($P < 0.001$) effect of genotype (G) and environment (E) on chickpea seed constituent traits (Table 5.1). The interaction between genotype and environment (G × E) also exhibited a significant effect ($P < 0.001$) on these chickpea seed constituent traits in RILs. A similar effect of G and E was observed on parents. In parents, protein (0.93) showed the highest $H^2$ followed by seed weight (0.68), total starch (0.27) and amylose (0.29), respectively. In RILs, maximum $H^2$ was observed for seed weight (0.65) followed by protein (0.57), total starch (0.29) and amylose (0.11), respectively (Table 5.2).

### 5.4.4 Construction of the intra-specific linkage map

The GBS assay identified a total of 822 variable SNPs. After filtering, 415 SNPs were identified and utilized for linkage mapping (Appendix 8). These markers were anchored on eight linkage groups (LG; Figure 5.3) that spanned for 662 cM of total map length with an average inter-marker distance of 1.60 cM (Table 5.3). LG1 is the largest linkage group with 185 markers having total map length and average marker distance of 125 and 0.67 cM, respectively.

### 5.4.5 QTL analysis

QTL analysis for selected chickpea seed constituent traits was performed using Icimapping version 4.1 and MapQTL version 5 with the same parameters. Both software packages identified the same QTLs for all selected traits. QTLs obtained from Icimapping version 4.1 are reported.

#### 5.4.5.1 QTLs identified in each growing environment

The LOD thresholds were 2.7 for TSW, total starch, protein and amylose (Appendix 9). At the cut-off threshold for TSW, four and three QTLs were found in F 2011 and F 2013, respectively; whereas, two QTLs were identified for both F 2012 and G 2012. Phenotypic variance explained (PVE) values for these QTLs ranged from 4.0 to 23.2 % (Table 5.4). QTLs present on LG III and VI were consistent across all environments. Conversely, only one QTL located on LG III, was detected for total starch. The QTL was common in all four growing locations and explained about 6.0 – 30.0 % of phenotypic variance in each environment (Table 5.4). In F 2011 and G 2012, two QTLs on LG III and LG VI were linked to protein

**Table 5.1** Analysis of variance (ANOVA) with F values for selected seed constituent traits.

| Type | Effect | numDF | F values | | | |
|---|---|---|---|---|---|---|
| | | | TSW | Total starch | Protein | Amylose |
| Parents | E | 3 | 19.70** | 21.07** | 8.58* | 458.47*** |
| | G | 1 | 221.17*** | 151.52*** | 495.62*** | 30.32*** |
| | G × E | 3 | 20.22*** | 59.94*** | 6.56** | 9.55*** |
| | CV (%) | | 44.15 | 11.13 | 20.70 | 13.04 |
| Offspring | E | 3 | 108.61*** | 22.42** | 119.18*** | 3335.53*** |
| | G | 221 | 150.66*** | 23.11*** | 70.47*** | 27.35*** |
| | G × E | 663 | 15.36*** | 7.62*** | 8.49*** | 17.95*** |
| | CV (%) | | 37.90 | 15.39 | 16.18 | 10.73 |

*, ** and *** are significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$. E, Environment; G, Genotype.

**Table 5.2** Estimates of variance components and broad-sense heritability ($H^2$) for selected constituent traits.

| Type | Variance component | TSW | Total starch | Protein | Amylose |
|---|---|---|---|---|---|
| Parents | $\sigma_g^2$ | $2.88\times10^3\pm4.49\times10^3$ | 7.67±18.41 | 26.56±38.07 | 1.16±2.43 |
| | $\sigma_{gl}^2$ | $1.10\times10^3\pm9.46\times10^2$ | 19.74±16.39 | 1.21±1.17 | 1.93±1.74 |
| | $\sigma_e^2$ | $2.29\times10^2\pm72.49$ | 1.34±0.42 | 0.87±0.28 | 0.80±0.23 |
| | $\sigma_p^2$ | $4.21\times10^3$ | 28.75 | 28.64 | 3.89 |
| | $H^2$ | 0.68 | 0.27 | 0.93 | 0.29 |
| Offspring | $\sigma_g^2$ | $1.55\times10^3\pm1.64\times10^2$ | 9.58±1.38 | 3.37±0.37 | 0.53±0.14 |
| | $\sigma_{gl}^2$ | $6.59\times10^2\pm38.75$ | 16.32±1.03 | 1.63±0.10 | 3.36±0.20 |
| | $\sigma_e^2$ | $1.83\times10^2\pm23.49$ | 9.89±0.27 | 0.87±0.02 | 0.79±0.02 |
| | $\sigma_p^2$ | $2.39\times10^3$ | 35.79 | 5.87 | 4.68 |
| | $H^2$ | 0.65 | 0.27 | 0.57 | 0.11 |

$\sigma_g^2$ = genotypic variance; $\sigma_{gl}^2$ = genotype × location interaction variance; $\sigma_e^2$ = error variance; $\sigma_p^2$ = phenotypic variance.

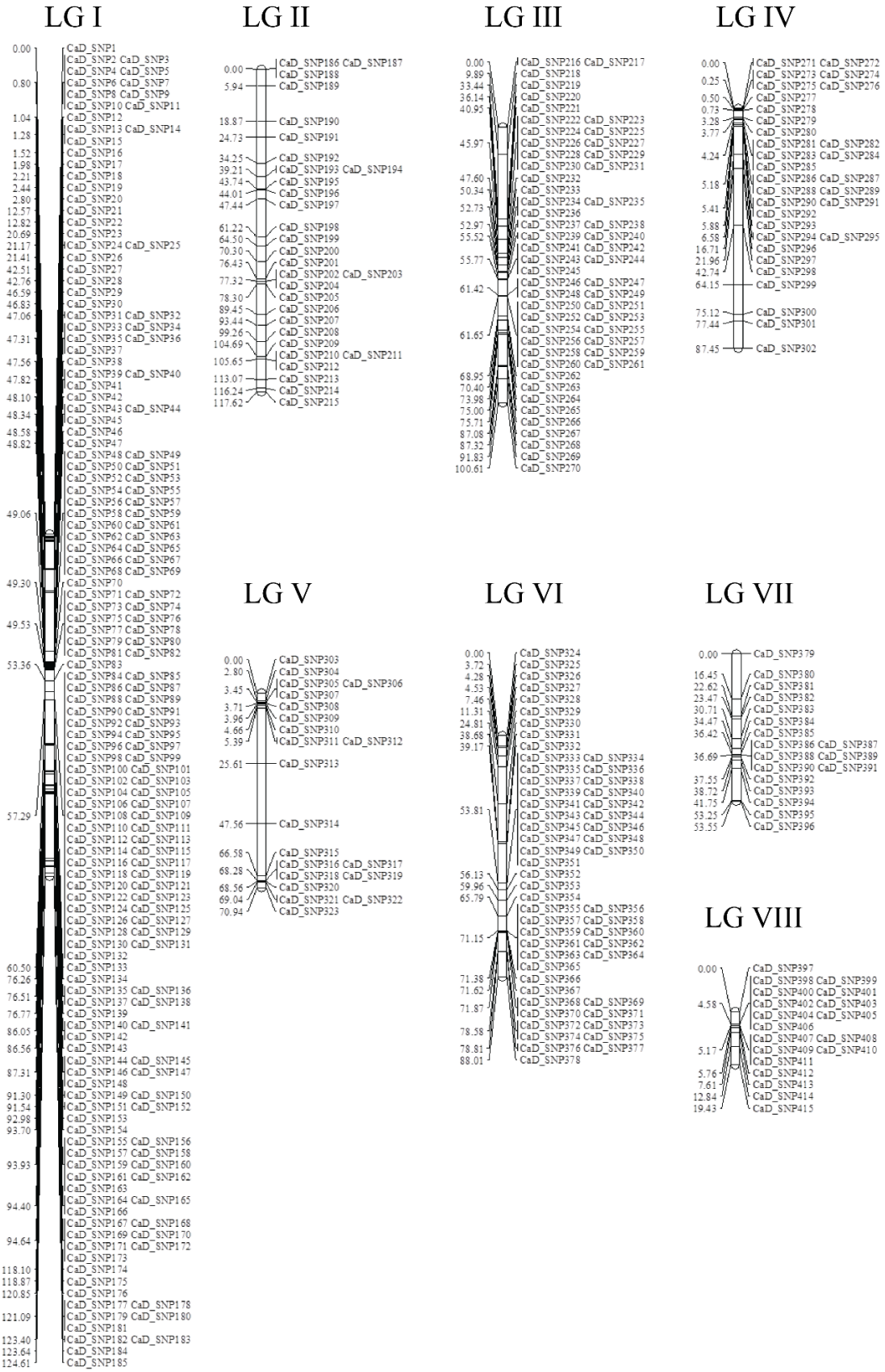


**Figure 5.3** Genetic linkage map based on 415 SNP markers segregating in 222 RILs derived from an intra-specific cross between two desi chickpea genotypes, ICC 995 × ICC 5912 constructed using the desi reference genome-based GBS assay.
Marker name is on the right side of the linkage group (LG) where as their corresponding position (cM) is on the left side.

**Table 5.3** Distribution of SNP markers on linkage groups.

| Linkage group | Mapped length (cM) | No. of markers | Average marker distance (cM) |
|---|---|---|---|
| I | 124.61 | 185 | 0.67 |
| II | 117.62 | 30 | 3.92 |
| III | 100.61 | 55 | 1.83 |
| IV | 87.45 | 32 | 2.73 |
| V | 70.94 | 21 | 3.38 |
| VI | 88.01 | 55 | 1.60 |
| VII | 53.55 | 18 | 2.98 |
| VIII | 19.43 | 19 | 1.02 |
| Total | 662.22 | 415 | 1.60 |

**Table 5.4** QTLs identified for selected seed constituent traits in different environments.

| Trait | Locations[a] | LG | Position (cM)[b] | Left marker[c] | Right marker[c] | LOD | PVE (%) | Add[d] |
|---|---|---|---|---|---|---|---|---|
| TSW | F 2011 | I | 2.8 | CaD_SNP19 | CaD_SNP20 | 3.08 | 4.03 | 7.92 |
| | | I | 93.9 | CaD_SNP154 | CaD_SNP155 | 3.58 | 4.72 | 8.59 |
| | | III | 0.0 | CaD_SNP217 | CaD_SNP218 | 8.14 | 11.25 | 13.36 |
| | | VI | 78.3 | CaD_SNP371 | CaD_SNP372 | 11.92 | 17.64 | 17.06 |
| | G 2012 | III | 0.0 | CaD_SNP217 | CaD_SNP218 | 7.03 | 9.25 | 22.77 |
| | | VI | 77.8 | CaD_SNP371 | CaD_SNP372 | 14.97 | 23.17 | 36.80 |
| | F 2012 | III | 0.0 | CaD_SNP217 | CaD_SNP218 | 3.59 | 6.04 | 7.61 |
| | | VI | 77.8 | CaD_SNP371 | CaD_SNP372 | 6.34 | 11.76 | 10.82 |
| | F 2013 | I | 29.4 | CaD_SNP26 | CaD_SNP27 | 7.88 | 15.63 | 17.90 |
| | | III | 0.0 | CaD_SNP217 | CaD_SNP218 | 3.58 | 4.38 | 9.55 |
| | | VI | 78.5 | CaD_SNP371 | CaD_SNP372 | 13.41 | 18.39 | 19.95 |
| Total starch | F 2011 | III | 0.0 | CaD_SNP217 | CaD_SNP218 | 5.95 | 12.13 | 1.33 |
| | G 2012 | III | 0.0 | CaD_SNP217 | CaD_SNP218 | 15.96 | 30.02 | 2.98 |
| | F 2012 | III | 0.0 | CaD_SNP217 | CaD_SNP218 | 2.89 | 5.97 | 1.43 |
| | F 2013 | III | 0.0 | CaD_SNP217 | CaD_SNP218 | 7.81 | 15.16 | 1.41 |
| Protein | F 2011 | III | 0.0 | CaD_SNP217 | CaD_SNP218 | 11.61 | 14.53 | -1.05 |
| | | IV | 73.5 | CaD_SNP299 | CaD_SNP300 | 2.89 | 3.86 | -0.54 |
| | | VI | 77.6 | CaD_SNP371 | CaD_SNP372 | 14.72 | 20.90 | -1.29 |
| | G 2012 | III | 0.0 | CaD_SNP217 | CaD_SNP218 | 6.72 | 9.98 | -0.71 |
| | | VI | 76.7 | CaD_SNP371 | CaD_SNP372 | 8.29 | 14.07 | -0.86 |
| | F 2012 | II | 39.2 | CaD_SNP192 | CaD_SNP193 | 2.94 | 4.63 | -0.51 |
| | | VI | 78.2 | CaD_SNP371 | CaD_SNP372 | 6.51 | 10.45 | -0.81 |
| | F 2013 | VI | 77.3 | CaD_SNP371 | CaD_SNP372 | 14.65 | 25.12 | -1.21 |
| Amylose | F 2011 | VI | 38.6 | CaD_SNP330 | CaD_SNP331 | 3.38 | 6.84 | 0.83 |
| | G 2012 | Undetected | | | | | | |
| | F 2012 | III | 0.0 | CaD_SNP217 | CaD_SNP218 | 4.87 | 7.62 | -0.35 |
| | F 2013 | II | 96.3 | CaD_SNP207 | CaD_SNP208 | 3.86 | 7.21 | -0.29 |
| | | III | 0.0 | CaD_SNP217 | CaD_SNP218 | 5.60 | 9.08 | -0.33 |
| | | VI | 42.8 | CaD_SNP332 | CaD_SNP333 | 4.25 | 8.48 | 0.31 |

[a] The mapping population was grown in four environments at ICRISAT (F 2011), Biggar (F 2012), agricultural greenhouse (G 2012) and Aberdeen (F 2013).
[b] the position of the LOD peak.
[c] the markers flanking the position of the LOD peak.
[d] positive values indicate alleles are donated by ICC 995, whereas negative values indicate alleles are donated by ICC 5912.

concentrations in RILs; whereas, a QTL on LG VI was also detected in F 2012 and F 2013 environments. The common QTL among all the environments, located on LG VI, explained 10.5 – 25.1 % phenotypic variance for protein. No consistent QTL across all environments was observed for amylose concentration. One QTL each in F 2011 and F 2012 was detected on LG VI and III, respectively. In F 2013, three QTLs were detected for amylose concentration on LG II, III and VI. However, no QTL was found for amylose concentration in G 2012. These QTLs had a minor effect on the trait, as it explained less than 10 % of phenotypic variance.

#### 5.4.5.2 Common QTLs detected in multiple environment trials

The LOD threshold values were 4.3, 4.3, 4.4 and 4.3 for TSW, total starch, protein and amylose, respectively. LOD values for each trait were analyzed in total and separately for genotype and its interaction with environment (Appendix 10). QTL analysis with multiple-environmental trial (MET) method utilized pooled data from all environments and detected a total of eight QTLs: three on LG I, one each on LG II, III and IV and two on LG VI. The consistent QTLs in MET and individual environmental test were summarized in Figure 5.4.

QTL located on LG III was associated with all chickpea seed constituent traits explaining 10.7, 18.6, 9.3 and 8.3 % of phenotypic variance for seed weight and concentrations of total starch, protein and amylose, respectively. Another major QTL located on LG VI was mainly linked to seed weight and protein concentration, and it explained 24.6 and 19.4 % of phenotypic variance, respectively. Minor QTLs for the traits explained only 2.5 to 4.4 % of the phenotypic variance. Notably, the LOD values contributed by the QTL and environment interaction were very low for all the traits except amylose (Table 5.5).

#### 5.4.5.3 Epistatic QTLs identified for seed storage components

Analysis of the epistasis events for QTLs governing the four seed constituent traits, *i.e.* TSW, total starch, protein and amylose, was also conducted for the multiple environment trials. A total of 13 pairs of QTLs interacting with each other were detected for TSW but explained only 0.6 – 4.7 % of total phenotypic variation. Six pairwise QTLs showed epistatic interaction for total starch concentration, explaining a phenotypic variation of 2.5 %, 2.4 % and 2.7 %, respectively. Protein concentration had 12 pairs of QTLs with epistatic effects attributed for 1.8 – 4.4 % of phenotypic variation. As for amylose, 14 QTLs had high marker-trait associations and the epistatic interactions with each other, explaining 0.9 – 2.8 % of total variation. The LOD values caused by the additive × additive effects were high for TSW and

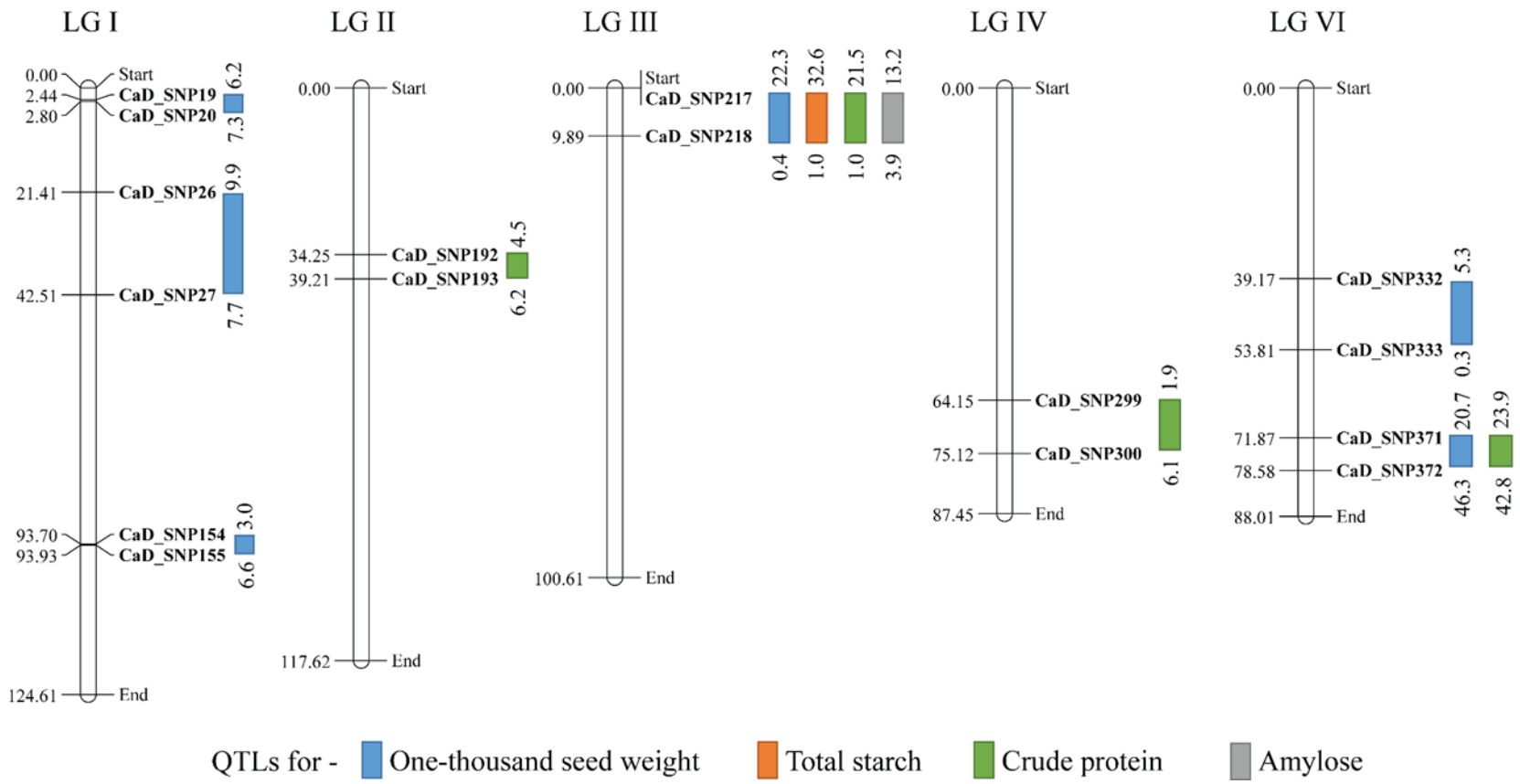


**Figure 5.4** Linkage groups (LGs) showing the location (in cM and on left side of LG) of SNP markers (right side of LG) linked to the most consistent QTLs for selected chickpea seed constituent traits.
The LOD values of the left and right marker of the QTLs were placed on the corresponding positions.

**Table 5.5** QTLs identified through multiple-environment trials (MET).

| Trait | LG | Position (cM)[a] | Left marker[b] | Right marker[b] | LOD | LOD (A)[c] | LOD (AbyE)[d] | PVE (%) | PVE (A) (%)[c] | PVE (AbyE) (%)[d] | Add[e] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| TSW | I | 2.8 | CaD_SNP19 | CaD_SNP20 | 7.32 | 5.87 | 1.45 | 2.74 | 2.37 | 0.37 | 6.79 |
| | I | 28.9 | CaD_SNP26 | CaD_SNP27 | 11.45 | 8.92 | 2.52 | 4.40 | 3.68 | 0.72 | 8.49 |
| | I | 93.9 | CaD_SNP154 | CaD_SNP155 | 6.64 | 5.48 | 1.16 | 2.59 | 2.22 | 0.38 | 6.58 |
| | III | 0.0 | CaD_SNP217 | CaD_SNP218 | 22.34 | 20.33 | 2.01 | 10.65 | 8.94 | 1.72 | 13.32 |
| | VI | 39.2 | CaD_SNP332 | CaD_SNP333 | 5.35 | 5.09 | 0.26 | 2.52 | 2.03 | 0.48 | -6.29 |
| | VI | 78.3 | CaD_SNP371 | CaD_SNP372 | 46.46 | 41.87 | 4.59 | 24.56 | 20.58 | 3.99 | 20.62 |
| Total starch | III | 0.0 | CaD_SNP217 | CaD_SNP218 | 32.62 | 27.98 | 4.64 | 18.62 | 16.21 | 2.41 | 1.79 |
| Protein | II | 39.2 | CaD_SNP192 | CaD_SNP193 | 6.24 | 6.01 | 0.23 | 2.56 | 2.36 | 0.20 | -0.34 |
| | III | 0.0 | CaD_SNP217 | CaD_SNP218 | 21.47 | 18.54 | 2.93 | 9.29 | 7.55 | 1.75 | -0.61 |
| | IV | 75.1 | CaD_SNP299 | CaD_SNP300 | 6.15 | 5.90 | 0.25 | 2.49 | 2.32 | 0.17 | -0.34 |
| | VI | 77.6 | CaD_SNP371 | CaD_SNP372 | 43.78 | 42.74 | 1.04 | 19.38 | 18.72 | 0.65 | -0.99 |
| Amylose | III | 0.0 | CaD_SNP217 | CaD_SNP218 | 13.18 | 7.09 | 6.09 | 8.25 | 7.91 | 0.34 | -0.37 |

[a] the position of the LOD peak.

[b] the markers flanking the position of the LOD peak.

[c] LOD or PVE caused by the additive effect.

[d] LOD or PVE caused by the interaction of the additive effect with the environment.

[e] positive values indicate alleles are donated by ICC 995, whereas negative values indicate alleles are donated by ICC 5912.

protein, whereas for total starch and amylose the values were low resulting from a relatively high LOD values caused by the interactions of additive × additive effects with environment (Appendix 11 and 12).

#### 5.4.5.4 Candidate gene analysis

One QTL, explaining a higher phenotypic variance for all the selected seed constituent traits, had two flanking markers (CaD_SNP217 and CaD_SNP218) but on different chromosomes. However, only one (CaD_SNP217) of the markers showed a significantly higher LOD value was located on chromosome 1 in a gene coding for COBRA-like protein 6-like protein (COBL-6-like; UniRef90_UPI00032A5E50). Two QTLs had flanking markers on the same chromosomes 3 and 4. QTL on chromosome 3 explained only 1.9 – 2.4 % of phenotypic variation in seed weight and contained four genes out of which protein disulfide-isomerase-like protein (UniRef90_UPI00032A7B61) coding gene might be a putative candidate gene. A major QTL for seed weight and protein was detected on chromosome 3 flanked by CaD_SNP371 and CaD_SNP372. The QTL contained 877.1 kbp coding a total of 105 proteins (71 characterized and 34 uncharacterized). Most of these proteins were membrane- (25 %), cytosol/cytoplasm- (14 %) and nucleus- (13 %) associated. The proteins were assigned mainly for catalytic (44 %) and binding (44 %) functions in various biological processes (Appendix 13). Genes coding for pentatricopeptide repeat-containing protein (PPR proteins; UniRef90_UPI00032ACF49), Acyl-CoA-binding domain-containing protein (ACBP; UniRef90_A2Q340), cyclin-dependent kinase 12-like protein (CDK 12-like protein; UniRef90_UPI00032A63F5), sucrose non-fermenting 4-like protein-like isoform (SnRK-like protein; UPI00032A9051) and proton-coupled amino acid transporter 3-like isoform (PAT 3-like protein; UPI00032ABECC) might be the putative candidates for this QTL. Protein BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) of PAT 3-like protein identified a conserved protein domain SdaC (amino acid permease; COG0814) in the sequence.

## 5.5 Discussion

Natural variation for seed constituent traits among RILs was characterized in the present study through multiple environmental experiments. RILs showed a wide variation for all seed constituent traits studied, thus making it an important mapping population for QTL studies. The present study established a significant effect of G, E and G × E on seed constituent traits in chickpea. Significant effects of G, E and G × E have been reported previously on chickpea seed constituents like soluble sugars (Gangola et al., 2013), starch (Frimpong et al.,

2009) and protein (Alwawi et al., 2010). Seed size was significantly influenced by G, E and G × E in soybean (*Glycine max*; Jaureguy et al., 2011). The study revealed the correlations among TSW and concentrations of total starch, protein and amylose that concurred with the results of Frimpong et al. (2009), Ozer et al. (2010) and Xu et al. (2014). A study of major seed constituents in lentils reported a positive correlation between starch and TSW; in contrast to negative correlation of amylose to TSW and total starch (Tahir et al., 2011).

The criterion of heritability can be impacted by several factors including genetic background of the plant materials used, population types, environmental influence and experimental design (Meng et al., 2016). A strong heritability estimate of a trait is the key for a successful identification of QTLs that govern the trait (Meng et al., 2016). Broad sense heritability has been categorized as high (> 0.60), medium (0.60 – 0.30), and low (< 0.30) heritability (Gangola et al., 2013). Seed weight was established as highly heritable trait followed by protein and total starch; whereas, amylose is the least heritable trait. High heritability of seed weight and protein concentration in chickpea is in agreement with the conclusions of Malik et al. (2010). Low repeatability of total starch and amylose concentrations in chickpea supports the low heritability of these traits in the present study (Frimpong et al., 2009).

Composite interval mapping (CIM) approach is the most prevalent tool to identify QTL (Zeng, 1994). Compared with simple interval mapping, CIM reduces the multiple dimensional search for QTLs to a one-dimensional search through restricting the search in one region every time, and promotes the precision and power of detecting QTLs when markers linked to other QTLs are taken into consideration. CIM also elevates the efficiency of QTL analysis with the simultaneous estimation of multiple markers (Zeng, 1994). However, the weakness of the CIM algorithm in the handling of background marker variables could bring about a biased estimation of the QTL effect under investigation (Li et al., 2007; Wang, 2009). This weakness can be overcome by the inclusive composite interval mapping (ICIM) method described by Wang (2009). ICIM method also prevents potential sampling variances; therefore, it was utilized to discover QTLs in the present study.

ANOVA established a significant effect of G×E on all chickpea seed constituent traits. QTL analysis with pooled/mean data from all environments could lead to type I or type II error. Therefore, QTL analysis was executed in different environments separately for all seed constituent traits resulting in both common/consistent and environment specific or inconsistent QTLs. Consistent QTLs also had different LOD values, explained phenotypic variances and additive effects in different environments thus confirming the significant effect of environment

on phenotypic expression and genetic control of chickpea seed constituent traits, *i.e.* QTL × environment interaction (QEI) that prevails for seed constituent traits (El-Soda et al., 2014). To explore this interaction, the MET method available in QTL Icimapping v4.1 was utilized. MET analysis confirmed the stability of QTLs and assigned the LOD and PVE to genetic and environmental factors (Li et al., 2007). The estimation of positions and effects of QTLs with multi-environment phenotypic data from all locations also avoids errors with single-environment trial as it processes whole phenotypic data simultaneously (Li et al., 2007). It is important to note that some QTLs with major or minor effects were identified for the selected traits irrespective of single- or multi-environment analysis. The commonly detected genomic regions in the present study are considered as robust QTLs that should be studied in the future.

Epistatic interactions between non-allelic loci for seed constituent traits in chickpea were also investigated in the present study. Additive × additive interaction at different loci provides a tool to elucidate genetic effects on the expression of a phenotype (Bocianowski, 2013). The inclusion of epistatic interactions at other loci to the identification of QTLs assists in a comprehensive estimation of all the possible QTLs (Bocianowski, 2013). Different pairs of interacting loci, detected in this study, were of minor effects and absent in single- and multi-environment analyses. No interaction was observed for the robust QTLs. These findings indicated that the interacting loci unveiled in this analysis have no function alone on seed constituent traits, but they contribute to the phenotype through pairwise interactions.

Genetic studies on extrinsic seed constituent traits in chickpea, such as seed weight, are well documented. Many researchers reported distinct QTLs using different types of markers and mapping populations (Hossain et al., 2010b; Vadez et al., 2012; Das et al., 2015). Therefore, no consistency was observed among these reports in terms of location and phenotypic effect. The QTLs identified in the present study are novel for chickpea seed weight and concentrations of total starch, protein and amylose. The study is the first to report the QTL analysis for the latter three traits in chickpea including the effects of QTL × environment interaction (QEI) and epistasis on the phenotype. Some intriguing QTLs also showed significant impact on more than one trait, *e.g.* two QTLs, on LG III and VI with major effects on TSW, were also linked to total starch and protein. This might be due to pleiotropy or correlation among the traits. Starch and protein, as the two major seed components, constitute approximately 70 % of dry seed weight. They are highly correlated with each other and to seed weight. Amylose is one of the two major constituents of starch and therefore, highly correlated to it. These correlations may be responsible for the common QTLs detected among different seed constituent traits. It is also worth noting: (i) that the alleles contributing to TSW and total starch, which were positively

correlated, were contributed by ICC 995 (parent with high seed weight and total starch, but low in protein and amylose), (ii) that protein and amylose, also positively correlated, obtained the alleles from ICC 5912 (the other parent with low seed weight and starch, but high protein and amylose), and (iii) that the traits with negative correlations that had common QTLs inherited the alleles from different parents.

The putative genes identified in the present study have been previously characterized for their function in a plant cell. COBL-6-like proteins, extracellular glycosyl-phosphatidyl inositol-anchored protein, co-express with *CesA* genes that code the catalytic subunit of cellulose synthase and therefore, regulate the expansion and deposition of cellulose microfibrils in seed coat (Roudier et al., 2005; Ben-Tov et al., 2015). However, the function of COBL-6-like proteins in cellulose biosynthesis has not been reported to-date. Protein disulfide-isomerase-like protein-family has been characterized to regulate amount and composition of seed proteins thus influencing the endosperm development in rice (Kim et al., 2012). Seed coat and endosperm are essential components of a legume seed and affect its final size/weight (Weber et al., 2005). PPR protein participates in seed development, various biosynthetic processes and defense responses (Barkan and Small, 2014) whereas, ACBP is important for fat metabolism and embryo development (Xiao and Chye, 2011). CDK 12-like protein regulates cell division which is important for seed development (Tank and Thaker, 2011). SnRK is an important family of protein kinases described as global regulator of carbon metabolism, and therefore, influence accumulation of seed storage compounds in pea (Radchuk et al., 2006). PAT 3-like protein is a strong candidate to modulate protein concentration in legume seeds. It is an amino acid transporter and has been attributed to regulate storage protein synthesis in *Vicia faba* L. (Miranda et al., 2001; Weber et al., 2005).

# CHAPTER 6. GENERAL DISCUSSION AND CONCLUSIONS

In the last few years, chickpea (*Cicer arietinum* L.) has been the second most produced pulse crop around the world (FAOSTAT, 2014). The major focus of chickpea improvement has been on disease resistance or environmental stresses to improve agronomic performance and grain yield (Gaur et al., 2007). In the last few years, improvement of chickpea grain quality has also attracted the attention of some researchers (Jukanti et al., 2012). To develop genetic strategies to improve grain quality, the first step is to identify chickpea genotypes with desired seed composition. The second step is to evaluate the stability of desired seed composition under diverse environments. The third step is to determine the genetic basis of variation and develop genetic markers to accelerate the development of cultivars with desired seed composition and quality. Those were the objectives of the work described in the three preceding chapters. The work was based on the hypothesis that: 1) chickpea genotypes show natural variation for the selected seed composition traits, and 2) the natural variation is associated with specific genomic regions in chickpea.

## 6.1 Phenotypic evaluation and identification of genotypes with good seed constituent traits

To characterize natural variation for seed composition traits, three types of germplasm collections were used. In the first study (Chapter 3), a reference set that is a subset of the core collection, in the second study a composite collection developed for seed constituent traits, essentially based on visual characteristics (Chapter 4) and in the third study a RIL population derived from parents that showed large variation in protein concentration (Chapter 5), were evaluated. The reference set is based on geographic distribution, and therefore, genotypes from the center of origin (Syria and Turkey) showed the highest diversity as determined by SDI (Table 3.1). The TSW, total starch and amylose concentration showed higher SDI in the genotypes from the primary center of origin compared to the secondary center of origin (Table 3.1). Protein concentration did not follow the trend observed for the other three traits. The results concur with previous reports (Engels et al., 2006) that there is higher natural variation at the center of origin as compared to the primary and secondary center of distribution. The composite collection that contained genotypes from the primary and secondary center of distribution did not show much variation in SDI (0.43 to 0.65) between the regions (Table 4.5). In the RIL population, TSW showed the lowest SDI (0.63), but the SDI (0.73 to 0.78) in the

three other traits, protein and starch and amylose concentrations was similar (Table 6.1).

The RIL population was developed from a cross between ICC 995 × ICC 5912 with 16.8 % and 24.0 % of protein, respectively. The TSW and starch concentrations were higher in ICC 995 and ICC 5912. An interesting observation comparing the three different populations is that based on SDI similar degree of diversity is observed (Table 6.1).

Generally, in all chickpea populations investigated in the present study, a positive correlation was observed for TSW and total starch, and it was also observed for protein and amylose. However, TSW and total starch had a negative correlation with protein and amylose. The negative correlations among the seed constituent traits complicate the direct selection for a trait of interest.

Influence of genotype and environment interaction (G × E) has been widely studied on agronomical traits of chickpea. However, the effects of G×E interaction on seed constituent traits have been studied in only a few cases (Frimpong et al., 2009). In chickpea, significant G × E interaction was observed for raffinose family oligosaccharides (RFOs) and these traits also showed low to medium broad sense heritability (0.25 – 0.56) (Gangola et al., 2013).

In the reference, composite and RIL collections, high (≥ 0.6) broad sense heritability was observed for thousand seed weight (Table 6.2). The protein concentration showed medium level of broad sense heritability in the RIL population, while it was high in the parents. All of the other seed composition traits, in general, showed low level of broad sense heritability (Table 6.2). The low heritability estimates were due to high G × E effect, reflecting a lower consistent agronomical performance of the seed composition traits. However, heritability estimates are affected by several factors. The genetic background of the plant materials used, population types, environmental influence and experimental design are some of the factors that impact heritability (Meng et al., 2016). A strong heritability suggests the high consistency of performance of a trait between environments, and therefore, the ease of selection of superior genotypes. Low heritability usually results from large effect of G × E and it complicates the selection of appropriate genotypes for variety development (Romagosa and Fox, 1993). Based on the studies reported in this thesis, three desi accessions, ICC 16903, ICC 4958 and ICC 93954, two kabuli accessions, ICC 7255 and ICC 8261, and one pea-shaped accession, ICC 8350 showed desirable seed composition and consistent performance across the environments (Table 6.3). The identified genotypes can be utilized in breeding programs depending on different end-use goals.

**Table 6.1** Shannon-Weaver diversity index (SDI) for selected seed constituent traits in the chickpea germplasm collections and RILs.

| Traits | SDI | | |
|---|---|---|---|
| | Reference set | Composite collection | RILs |
| TSW | 0.68 | 0.77 | 0.63 |
| Protein | 0.64 | 0.74 | 0.77 |
| Starch | 0.77 | 0.71 | 0.73 |
| Amylose | 0.81 | 0.71 | 0.78 |

**Table 6.2** Broad sense heritability ($H^2$) for selected seed constituent traits in chickpea of the three sample populations.

| Trait | Composite collection | | Reference set | | | RILs (desi) | |
|---|---|---|---|---|---|---|---|
| | Desi | Kabuli | Desi | Kabuli | Pea-shaped | Parents | Progeny |
| TSW | 0.87 | 0.72 | 0.70 | 0.74 | 0.73 | 0.68 | 0.65 |
| Total starch | 0.30 | 0.48 | 0.13 | 0.15 | 0.13 | 0.27 | 0.27 |
| Protein | 0.34 | 0.17 | 0.16 | 0.29 | 0.23 | 0.93 | 0.57 |
| Amylose | 0.12 | 0.16 | 0.17 | 0.14 | 0.11 | 0.29 | 0.11 |

**Table 6.3** Detailed information of selected chickpea genotypes with good seed constituent traits.

| Genotype | TSW (g) | Total starch (%) | Amylose (%) | Protein (%) | Botanical type | Origin | Biological status | Feature |
|---|---|---|---|---|---|---|---|---|
| ICC 16903 | 181.8±15.6 | 44.4±1.9 | 36.6±1.6 | 21.5±4.4 | Desi | India | Landrace | Pod borer resistant/drought tolerant |
| ICC 7255 | 316.8±30.3 | 51.2±4.8 | 34.3±3.9 | 23.2±2.2 | Kabuli | India | Landrace | Drought susceptible |
| ICC 8350 | 251.8±16.3 | 41.1±5.7 | 35.5±3.3 | 22.6±2.6 | Pea-shaped | India | Landrace | Drought tolerant |
| ICC 4958 | 342.6±32.5 | 43.4±5.5 | 33.7±1.5 | 20.5±3.8 | Desi | India | Advanced cultivar | Drought tolerant |
| ICC 93954 | 315.7±25.5 | 44.8±1.5 | 33.4±1.4 | 20.0±1.7 | Desi | India | Breeding line | Drought susceptible |
| ICC 8261 | 335.3±42.6 | 43.9±6.5 | 33.5±2.0 | 22.3±2.1 | Kabuli | Turkey | Landrace | Drought tolerant |

## 6.2 Genomic search for DNA regions associated with selected seed constituent traits

To understand the genetic basis of seed composition trait variation, two strategies were employed. A genome wide association study (GWAS) using the composite collection of 168 chickpea genotypes and a linkage mapping strategy using a designed bi-parental mapping population of 222 RILs.

### 6.2.1 Genome wide association study (GWAS) of selected seed constituent traits

The 168 chickpea accessions of a composite germplasm collection made up of 115 desi- and 53 kabuli- type chickpeas were subjected to genome analysis using DNA diversity array technology (DArT). Total 380 DArT markers with an average PIC value of 0.16 were used to explore genetic variation in the chickpea germplasm collection. Two distinct populations (clusters) were recognized, but these populations did not separate the kabuli- or desi-type chickpeas. The two populations were further subdivided in to four sub-populations in each population (Figure 4.4). However, within each population (cluster) the desi and kabuli-type were separated in to different sub-clusters (Figure 4.4). This suggests common origin of the two types of chickpeas (Varma Penmetsa et al., 2016).

Thirty-three MTA were found using the DArT analyses, with 19 in the desi- and 14 in the kabuli-type chickpeas (Table 4.7). It is interesting to note that no common MTA were found for desi- and kabuli-type chickpeas. In the desi-type the marker cpPb-677692 is associated with TSW, protein and starch concentration (Table 4.7). MTAs for seed weight are well documented in the literature (Kujur et al., 2014; Thudi et al., 2014; Bajaj et al., 2015; Kujur et al., 2015a; Kujur et al., 2015b), MTAs for seed composition such as total starch, protein and amylose are not reported to-date. Jadhav et al. (2015) reported MTAs in 168 chickpea accessions for protein using only 23 SSR markers. In the present study, MLM was chosen to perform the GWAS to eliminate false positives in detecting MTAs for the selected seed composition traits. The lack of nucleotide sequence of the DArT markers did not allow the precise location of these markers on the chickpea genome.

### 6.2.2 Bi-parental population based quantitative trait locus (QTL) mapping of selected seed constituent traits in multi-environment testing (MET)

A mapping population of 222 chickpea lines arising from the intra-specific cross of ICC 995 and ICC 5912, two desi accessions of Indian and Mexican backgrounds, respectively, and of a significant difference in protein, was analyzed by GBS. A total of 822 SNPs were identified by GBS. After removing the redundant SNPs with missing data, 415 informative SNPs were

used to detect MTA for seed composition traits. SNPs can assess a large number of loci in genomes, which increases their power compared with other marker systems (Foster et al., 2010). The co-dominant nature of SNPs increases its efficiency to distinguish homozygotes from heterozygotes (Arif et al., 2010). Above all, GBS based SNP discovery is a highly automated approach which allows efficient handling of numerous plant samples (Tsuchihashi and Dracopoli, 2002). Linkage mapping using SNP markers detected several QTLs with minor or major effects for the selected seed composition traits. Notably, some QTLs discovered in the present study were associated with more than one traits. For example, major QTLs for total starch and those for protein were also associated with TSW. One common QTL with major effect was found to be responsible for both total starch and amylose and also associated with protein. This might be because all the quality traits studied were highly correlated with one another. In addition, the observed phenomenon in the study also suggested a pleiotropic effect for these QTLs. On the other hand, high heritability for TSW, medium to low heritability for total starch, protein and amylose (Table 5.2) also contributed to the success of the QTL discovery, because strong heritability of a trait is the key for a successful identification of QTLs (Meng et al., 2015). The inclusive composite interval mapping (ICIM) method was implemented in QTL analysis to avoid a biased estimation of the QTL effect under investigation due to the limitations of CIM in the handling of background marker variables, and to aim at preventing potential sampling variance and tedious background marker selection and calculation (Li et al., 2007; Wang, 2009).

To understand the action of genomic regions, *i.e.* QTLs, in the dependence on the environment, QEI needs to be studied. The QTL analysis identified both common QTLs and environment-specific QTLs. Notably, all the detected QTLs showed varying QTL effects from environment to environment, which indicates the QEI (Mackay, 2001; MacMillan et al., 2006; Zhang et al., 2010a). The QEI effects reflect the magnitude of the robustness of the identified QTLs. Simply comparing the QTLs discovered in each location could cause biased QEI effects (Jansen et al., 1995; Tinker and Mather, 1995). The QTL analysis through MET found the QTLs associated with the selected seed composition traits, and meanwhile showed the contribution of the major additive effects and the QEI effects to the total LODs and total PVEs (Table 5.5). The estimation of position and effects of QTLs using multi-environment phenotypic data from all locations can avoid errors with single-environment trial because of the simultaneous processing of phenotypic data, increasing the statistical power to identify QTLs (Li et al., 2007).

To utilize QTLs efficiently, digenetic interactions between non-allelic loci for the selected seed constituent traits in chickpea have been rarely recorded. In this study 13 pairs of

interacting loci for TSW, three pairs for total starch, 12 pairs for protein and seven pairs for amylose content were identified (Appendix 11 and 12).

Among the seven candidate genes in the QTLs, PAT 3-like protein coding gene (UPI00032ABECC) is considered the most promising gene. It has been studied in faba bean and pea (Miranda et al., 2001; Weber et al., 2005; Zhang et al., 2015). However, in chickpea this gene has not been characterized yet. Therefore, the gene should be characterized to improve chickpea protein concentration and the overall chickpea seed quality.

### 6.2.3 Comparison of QTL mapping and GWAS of selected seed composition traits

Two methods, association mapping and linkage mapping were used to identify genomic regions associated with seed composition traits. Both the methods revealed genomic regions associated with seed composition traits. Linkage analysis study was successful and resulted in the identification of several QTLs. Some factors that ensured the success of the linkage analysis were parental lines showing distinct contrast in protein concentration, number of markers genotyped in whole genome, co-dominant property of SNP markers with informative polymorphisms and the QTL detecting method with high statistical power. Based on the marker sequences, some candidate genes were also identified. The GWAS was also useful, as the population structure and the kinship were taken into consideration when conducting the GWAS. Additionally, the GWAS was performed on desi and kabuli chickpeas separately in consideration of potential difference between desi and kabuli genomes, and therefore the chance of identifying valid trait associated genomic regions was enhanced. It is of interest to note both the GWAS and linkage mapping identified at least one set of markers that was common to TSW, protein and starch concentrations. Despite of the success of the study, results can be further improved by increasing the map resolution with more markers that can saturate the whole genome. Additionally, due to the lack of DArT marker sequences for chickpea, candidate genes could not be identified or located physically in the chickpea genome, and therefore the QTL analysis and the GWAS could not be combined.

## 6.3 Potential application of the identified genetic resources

Through the present study, we identified several chickpea genotypes from the three germplasm collection. The identified three desi accessions, two kabuli accessions and one pea-shaped accession that showed desired seed composition and a consistent performance can be used in chickpea improvement programs. From the RIL population, genotypes with increased protein, or increased amylose can be used to develop genotypes with increased protein or less

digestible starch. Based on their characteristics and different end-use purposes, the genotypes can play different roles in chickpea utilization. For example, chickpea has been used as an excellent source of protein and therefore chickpea starch and its nutritional value have not received enough attention. Chickpea starch can satisfy the needs for energy, and amylose of chickpea starch has a positive influence on human digestive health. Chickpea of relatively high starch, amylose and protein at the same time will be a better option for people who consume chickpea not only for protein but also for a more balanced nutrition. On the other hand, chickpeas with high TSW and starch can be used for calorie-deficient poor people in developing countries who need high carbohydrates to meet their energy requirements. Chickpeas of high protein and amylose can be used to develop functional foods in affluent regions. In all, seed quality depends on end uses. In the study, the identified chickpea genotypes with several seed constituent traits were identified. The identified chickpea genotypes along with molecular markers can be used to accelerate the development of chickpea varieties with desired seed constituent traits.

### 6.4 Conclusions

This is a first systematic study of chickpea seed composition traits such as thousand-seed weight (TSW), total starch, protein and amylose concentration to identify useful natural variation and its genetic basis. Some of the salient findings are as follow:

1. Variation in TSW, total starch, protein and amylose is present in natural chickpea germplasm.
2. Significant effects of genotype and environment (G × E) interaction were observed for the selected seed composition traits in chickpea.
3. Broad sense heritability was high for TSW, and was low to medium for the seed composition traits such as total starch, protein and amylose.
4. TSW and total starch were negatively correlated with protein and amylose indicating a compromise should be made when selecting for genotypes with desired seed composition.
5. Chickpea genotypes with desirable seed composition traits were identified.
6. MTAs were identified for TSW, total starch, protein and amylose for desi and kabuli chickpeas.
7. Common QTLs with major and minor effects were associated with TSW, total starch, protein and amylose.
8. Candidate genes were identified and need to be studied further.

9. The results support the hypothesis that natural variation of chickpea seed constituent traits exits, and that the natural variation is associated with specific regions of chickpea chromosomes.

### 6.5 Future work

The present study on the selected grain quality traits in chickpea has made some interesting findings that need to be pursued further to realize the complete potential of this work:

1. A thorough phenotypic evaluation should be carried out in the contemporary chickpea germplasm to search for genotypes with exploitable genetic variation for grain quality traits.
2. Concentrations of lipids and other nutrients need to be determined.
3. Hydrolytic analysis of starch and protein needs to be studied to study the bioavailability of proteins and realize the complete potential of chickpea carbohydrates.
4. The candidate genes suggested in this work need to be characterized and assess their functionality.

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# APPENDICES

**Appendix A** Effect of genotype (G), environment (E) and G × E on different agronomic traits in chickpea.

| Reference | Traits studied | No. of genotypes | No. of environments | Result |
|---|---|---|---|---|
| Malhotra et al. (1997) | Seed yield | 24 | 3 years, 1 site | G**, $S^{ns}$, G×S* |
| Nleya et al. (2002) | Seed weight | 13 kabuli | 17 | G**, G×E**, E** |
| | Hydration coefficient | | | G**, G×E**, E** |
| | Appearance | | | $G^{ns}$, G×E**, $E^{ns}$ |
| | Texture | | | G**, G×E**, E** |
| Berger et al. (2006) | Grain yield | 46 | 3 years, 7 sites | G×E* |
| Choudhary and Hague (2010) | Primary branches per plant | 42 | 2 | $G^{ns}$, G×E**, E** |
| | Secondary branches per plant | | | G**, G×E**, E** |
| | Grain yield per plant | | | G**, G×E**, E** |
| Bakhsh et al. (2011) | Grain yield | 16 | 2 years, 6 sites | $G^{ns}$, E***, G×$E^{ns}$ |
| Pande et al. (2013) | Ascochyta blight severity | 29 | 2 years, 9 sites | Y****, L****, Y×$L^{ns}$, G****, Y×G****, L×G**** |
| Sharma et al. (2013a) | Botrytis grey mould severity | 25 | 11 | G****, E**** G×E**** |
| Pushpavalli et al. (2015b) | Pod number | 10 | 2 years, 1 site | G***, T***, G×$T^{ns}$ |
| | Pod weight | | | G***, T***, G×$T^{ns}$ |
| | Seed number | | | G***, T***, G×$T^{ns}$ |
| | Seed weight | | | $G^{ns}$, $T^{ns}$, G×$T^{ns}$ |
| | Seed yield | | | G***, T***, G×T*** |
| | Flower time | | | G***, T***, G×$T^{ns}$ |

*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ****, $P<0.0001$; ns, not significant; E, environment; G, genotype; L, location; S, site; T, treatment; Y, year.

**Appendix B** Effect of genotype (G), environment (E) and G × E on selected seed constituent traits in chickpea.

| Reference | Traits studied | No. of genotypes | No. of environments | Result |
|---|---|---|---|---|
| Singh et al. (1983) | Protein | 47, 25 desi, 15 kabuli | 2 years, 7 sites | L**, C**, L×C$^{ns}$ |
| Frimpong et al. (2009) | Seed yield | 7 desi, 9 kabuli | 9 | G**, G×L**, L** |
| | Seed weight | | | G**, G×L**, L** |
| | Starch | | | G**, G×L**, L** |
| | Amylose | | | G**, G×L**, L** |
| | Protein | | | G**, G×L**, L** |
| Gangola et al. (2013) | myo-inositol | 168 | 3 | G***, E***, G×E*** |
| | Galactinol | | | G***, E***, G×E*** |
| | Glucose | | | G***, E***, G×E*** |
| | Fructose | | | G***, E***, G×E*** |
| | Sucrose | | | G***, E***, G×E*** |
| | Raffinose | | | G***, E***, G×E*** |
| | Stachyose | | | G***, E***, G×E*** |
| | Verbascose | | | G***, E***, G×E*** |
| | Total RFO | | | G***, E***, G×E*** |
| Ashokkumar et al. (2014) | Violaxanthin | 3 desi, 5 kabuli | 2 years, 4 sites | C***, Y$^{ns}$, L***, C×Y$^{ns}$, C×L***, C×Y×L$^{ns}$ |
| | Lutein | | | C***, Y$^{ns}$, L$^{ns}$, C×Y$^{ns}$, C×L$^{ns}$, C×Y×L$^{ns}$ |
| | Zeaxanthin | | | C***, Y***, L***, C×Y$^{ns}$, C×L$^{ns}$, C×Y×L$^{ns}$ |
| | β-Carotene | | | C***, Y$^{ns}$, L$^{ns}$, C×Y***, C×L$^{ns}$, C×Y×L$^{ns}$ |
| | Total carotene | | | C***, Y$^{ns}$, L$^{ns}$, C×Y$^{ns}$, C×L$^{ns}$, C×Y×L$^{ns}$ |

*, $P$<0.05; **, $P$<0.01; ***, $P$<0.001; ns, not significant; C, cultivar; E, environment; G, genotype; L, location; T, treatment; Y, year.

**Appendix C** Recent studies regarding QTL mapping in chickpea.

| Populations | Population size | QTLs/genes | Types of marker | Traits | Reference |
|---|---|---|---|---|---|
| | | | | **Resistance traits** | |
| FLIP84-92C × PI 599072 | 142 | 2 QTLs | Isozyme, RAPD, ISSR | Ascochyta blight resistance | Santra et al. (2000) |
| ICC 4958 × PI 489777<br>FLIP84-92C × PI 599072 | 130<br>142 | 2 QTLs | STMS | Ascochyta blight resistance | Rakshit et al. (2003) |
| ICC1 × Lasseter | 85 | 6 QTLs | RGA, STMS | Ascochyta blight resistance | Flandez-Galvez et al. (2003) |
| FLIP84-92C × PI 599072 | 206 | 2 QTLs | STMS | Ascochyta blight resistance | Tekeoglu et al. (2004) |
| ILC 72 × Cr 5-10 | 97 | 1 QTL | RAPD, ISSR, STMS | Ascochyta blight resistance | Cobos et al. (2006) |
| ILC 3279 × WR 315 | 106 | 2 QTLs | RAPD, SCAR, STMS | Ascochyta blight resistance | Iruela et al. (2006) |
| ICCV 96029 × CDC Frontier | 186 | 3 QTLs | SSR | Ascochyta blight resistance | Tar'an et al. (2007) |
| ICCV 04516 × ICC 4991<br>ICCV 10 × ICCV 04516 | 179<br>94 | 3 QTLs | SSR, EST | Ascochyta blight resistance | Kottapalli et al. (2009) |
| ICCV 2 × JG 62 | 126 | 3 QTLs | SSR | Botrytis grey mould resistance | Anuradha et al. (2011) |
| C 214 × WR 315<br>C 214 × ILC 3279 | 188 | 2 QTLs | SSR | Ascochyta blight and Fusarium wilt resistance | Sabbavarapu et al. (2013) |
| JG 62 × WR 315 | 94 | 5 QTLs | STMS, AFLP | Fusarium wilt resistance | Patil et al. (2014) |
| ICC 4958 × ICC 1882 | 232 | 23 genes | SNP | Drought tolerance | Kale et al. (2015) |
| ICCV 2 × JG 11 | 188 | 2 QTLs | SSR, SNP | Salinity tolerance | Pushpavalli et al. (2015a) |

**Appendix C** Recent studies regarding QTL mapping (Continued).

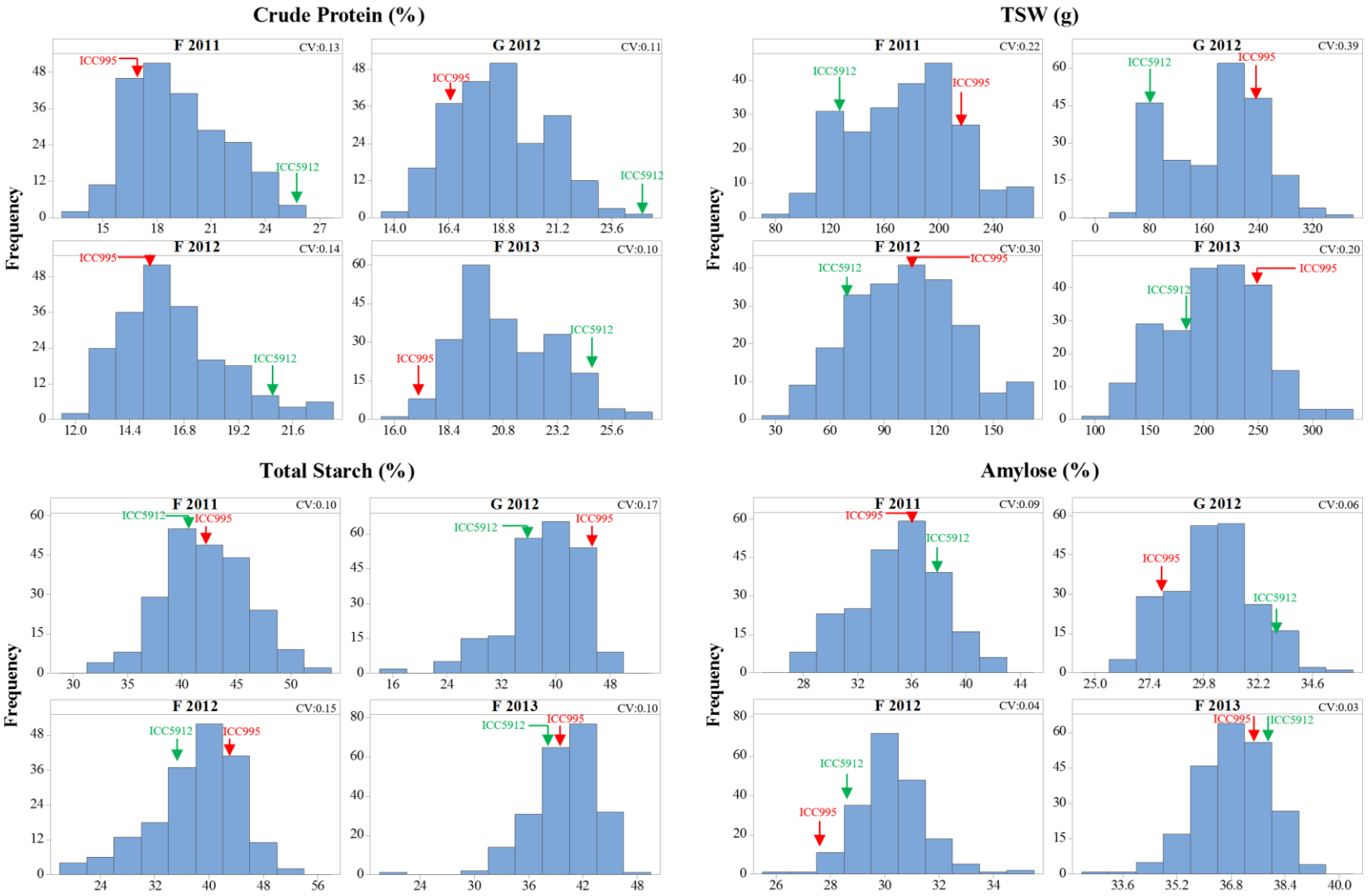
| Populations | Population size | QTLs/genes | Types of marker | Traits | Reference |
|---|---|---|---|---|---|
| | | | | **Agronomic traits** | |
| ICC 4918 × JM 2100 | 264 | 7 QTLs | RAPD | Leaf length, leaf width, erect plant habit | Banerjee et al. (2001) |
| ICCV 2 × JG 62 | 76 | 4 QTLs, 2 genes | STMS, RAPD, ISSR | Double podding, pigmentation, seed weight | Cho et al. (2002) |
| CA 2156 × JG 62 | 79 | 3 QTLs | ISSR, STMS, RAPD | Fusarium wilt resistance, flower color, double podding, seed coat thickness | Cobos et al. (2005) |
| CA2139 × JG 62 | 80 | | | | |
| CA 2156 × JG 62 | 80 | 3 QTLs | STMS, SSR | Seed size, yield, days to flowering | Cobos et al. (2007) |
| ICCL 81001 × Cr 5-9 | 88 | 6 QTLs, 2 genes | RAPD, ISSR, STMS | Seed coat thickness, flowering time, seed size, fusarium wilt resistance | Cobos et al. (2009) |
| ICC 3996 × S 95362 | 91 | 2 QTLs | SSR | Seed shape, stem color, flowering time | Hossain et al. (2010a) |
| S 95362 × Howzat | 105 | | | | |
| ICCV 2 × JG 62 | 126 | 2 QTLs | SSR | Seed yield under saline conditions | Vadez et al. (2012) |
| ICC 4958 × ICC 17160 | 190 | 6 QTLs | InDel | Seed number<br>Flowering and maturity time | Das et al. (2015) |
| ILC72 × Cr5-10 | 104 | 1 QTL, 2 genes | RAPD, SCAR | Growth habit | Ali et al. (2015) |
| WR315 × ILC3279 | 102 | | | | |
| ICC 6013 × ICC 7346 | 283 | 1 gene | SNP | Seed weight, pod | Kujur et al. (2015a) |
| | | | | **Seed constituent traits** | |
| Cr 205 × Hadas | 120 | 4 QTLs | STMS | β-carotene, lutein, seed weight, | Abbo et al. (2005) |

**Appendix D** Geographical origins and botanical types of chickpea genotypes used in study 1.

| Source | Desi | Kabuli | Pea-shaped | Total |
|---|---|---|---|---|
| Afghanistan | 7 | 4 | 3 | 14 |
| Algeria | 1 | 1 | 0 | 2 |
| Bangladesh | 1 | 0 | 0 | 1 |
| Chile | 0 | 1 | 0 | 1 |
| China | 0 | 1 | 0 | 1 |
| Cyprus | 1 | 0 | 0 | 1 |
| Egypt | 1 | 0 | 0 | 1 |
| Ethiopia | 13 | 1 | 0 | 14 |
| Greece | 1 | 0 | 0 | 1 |
| India | 75 | 4 | 4 | 83 |
| Iran | 50 | 18 | 0 | 68 |
| Israel | 0 | 2 | 0 | 2 |
| Italy | 3 | 0 | 0 | 3 |
| Malawi | 3 | 0 | 0 | 3 |
| Mexico | 2 | 2 | 0 | 4 |
| Morocco | 1 | 3 | 0 | 4 |
| Myanmar | 2 | 0 | 0 | 2 |
| Nepal | 2 | 0 | 0 | 2 |
| Pakistan | 5 | 0 | 0 | 5 |
| Peru | 0 | 1 | 0 | 1 |
| Portugal | 0 | 1 | 0 | 1 |
| Syria | 2 | 1 | 0 | 3 |
| Tanzania | 2 | 0 | 0 | 2 |
| Turkey | 5 | 3 | 1 | 9 |
| USSR | 2 | 3 | 0 | 5 |
| USA | 0 | 1 | 0 | 1 |
| Unknown | 1 | 2 | 0 | 3 |
| Total | 180 | 49 | 8 | 237 |

**Appendix E** Geographical origins and botanical types of chickpea genotypes used in study 2.

| Region | Desi | Kabuli | Total |
|---|---|---|---|
| Europe | 9 | 8 | 17 |
| Meso-America | 4 | 1 | 5 |
| North Africa | 9 | 10 | 19 |
| North America | 1 | 0 | 1 |
| South America | 0 | 2 | 2 |
| South Asia | 68 | 18 | 86 |
| Southwest Asia | 13 | 10 | 23 |
| Sub-Saharan Africa | 11 | 4 | 15 |
| Total | 115 | 53 | 168 |




**Appendix F** Phenotypic distribution of selected seed constituent traits in chickpea.
The red and the green arrows represent the two parents of the RILs, ICC 995 and ICC 5912, respectively.

**Appendix G** Selected RILs with consistent performance and higher values for selected seed constituent traits.

| Genotype | TSW (g) | | Starch (%) | | Amylose (%) | | Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| ICCRIL07-0002 | 222.7 | 54.7 | 43.7 | 2.1 | 33.5 | 2.9 | 18.6 | 2.2 |
| ICCRIL07-0149 | 251.6 | 59.5 | 44.9 | 3.0 | 33.9 | 4.7 | 16.4 | 1.7 |
| ICCRIL07-0170 | 227.6 | 70.3 | 43.8 | 5.4 | 34.0 | 5.0 | 16.5 | 1.9 |
| ICCRIL07-0184 | 241.1 | 69.9 | 41.8 | 8.0 | 33.0 | 3.0 | 18.1 | 1.5 |
| ICCRIL07-0198 | 89.6 | 34.3 | 30.8 | 8.9 | 34.8 | 3.7 | 22.1 | 2.3 |
| ICCRIL07-0201 | 240.7 | 58.3 | 46.3 | 4.5 | 34.2 | 3.2 | 17.0 | 2.8 |
| ICCRIL07-0203 | 97.8 | 54.5 | 28.0 | 10.1 | 36.9 | 3.4 | 23.9 | 2.3 |

**Appendix H** Detailed information of SNPs used for QTL analysis of selected seed constituent traits.

| SNP ID | Chromosome number | Physical position (bp) | Linkage group | Map position (cM) |
|---|---|---|---|---|
| CaD_SNP1 | scaffold00760 | 85540 | I | 0 |
| CaD_SNP2 | CaLG_4 | 368435 | I | 0.8 |
| CaD_SNP3 | CaLG_4 | 371754 | I | 0.8 |
| CaD_SNP4 | CaLG_4 | 348344 | I | 0.8 |
| CaD_SNP5 | CaLG_4 | 345799 | I | 0.8 |
| CaD_SNP6 | CaLG_4 | 338006 | I | 0.8 |
| CaD_SNP7 | CaLG_4 | 345941 | I | 0.8 |
| CaD_SNP8 | CaLG_4 | 368030 | I | 0.8 |
| CaD_SNP9 | CaLG_4 | 232426 | I | 0.8 |
| CaD_SNP10 | CaLG_4 | 241125 | I | 0.8 |
| CaD_SNP11 | CaLG_4 | 368237 | I | 0.8 |
| CaD_SNP12 | CaLG_4 | 223008 | I | 1.04 |
| CaD_SNP13 | CaLG_4 | 151462 | I | 1.28 |
| CaD_SNP14 | CaLG_4 | 198665 | I | 1.28 |
| CaD_SNP15 | CaLG_4 | 145927 | I | 1.28 |
| CaD_SNP16 | CaLG_4 | 123046 | I | 1.52 |
| CaD_SNP17 | CaLG_4 | 256346 | I | 1.98 |
| CaD_SNP18 | CaLG_4 | 348629 | I | 2.21 |
| CaD_SNP19 | CaLG_4 | 340829 | I | 2.44 |
| CaD_SNP20 | CaLG_4 | 367860 | I | 2.8 |
| CaD_SNP21 | CaLG_4 | 1248441 | I | 12.57 |
| CaD_SNP22 | CaLG_4 | 1248565 | I | 12.82 |
| CaD_SNP23 | CaLG_4 | 2391276 | I | 20.69 |
| CaD_SNP24 | scaffold01917 | 38145 | I | 21.17 |
| CaD_SNP25 | scaffold04579 | 17937 | I | 21.17 |
| CaD_SNP26 | scaffold04579 | 18040 | I | 21.41 |
| CaD_SNP27 | scaffold02445 | 12082 | I | 42.51 |
| CaD_SNP28 | scaffold00332 | 50967 | I | 42.76 |
| CaD_SNP29 | CaLG_4 | 4769476 | I | 46.59 |
| CaD_SNP30 | CaLG_4 | 4817421 | I | 46.83 |
| CaD_SNP31 | CaLG_4 | 4817665 | I | 47.06 |
| CaD_SNP32 | CaLG_4 | 4768373 | I | 47.06 |
| CaD_SNP33 | CaLG_4 | 4701677 | I | 47.31 |
| CaD_SNP34 | scaffold02142 | 26541 | I | 47.31 |
| CaD_SNP35 | scaffold03429 | 26838 | I | 47.31 |
| CaD_SNP36 | scaffold03429 | 8268 | I | 47.31 |
| CaD_SNP37 | scaffold00423 | 103678 | I | 47.31 |
| CaD_SNP38 | scaffold00423 | 61666 | I | 47.56 |
| CaD_SNP39 | scaffold00930 | 28419 | I | 47.82 |
| CaD_SNP40 | scaffold02142 | 30162 | I | 47.82 |
| CaD_SNP41 | scaffold00423 | 60386 | I | 47.82 |
| CaD_SNP42 | scaffold00930 | 91560 | I | 48.1 |
| CaD_SNP43 | CaLG_4 | 4556180 | I | 48.34 |
| CaD_SNP44 | CaLG_4 | 4690525 | I | 48.34 |
| CaD_SNP45 | CaLG_4 | 4701465 | I | 48.34 |
| CaD_SNP46 | CaLG_2 | 11393816 | I | 48.58 |

**Appendix H** Detailed information of SNPs used for QTL analysis of selected seed constituent traits (Continued).

| SNP ID | Chromosome number | Physical position (bp) | Linkage group | Map position (cM) |
|---|---|---|---|---|
| CaD_SNP47 | scaffold00991 | 91564 | I | 48.82 |
| CaD_SNP48 | scaffold00331 | 97468 | I | 49.06 |
| CaD_SNP49 | scaffold00331 | 97218 | I | 49.06 |
| CaD_SNP50 | scaffold00991 | 115253 | I | 49.06 |
| CaD_SNP51 | scaffold00331 | 119774 | I | 49.06 |
| CaD_SNP52 | scaffold00331 | 181554 | I | 49.06 |
| CaD_SNP53 | scaffold00331 | 181674 | I | 49.06 |
| CaD_SNP54 | scaffold00331 | 97359 | I | 49.06 |
| CaD_SNP55 | scaffold00906 | 26212 | I | 49.06 |
| CaD_SNP56 | scaffold00906 | 91967 | I | 49.06 |
| CaD_SNP57 | CaLG_7 | 1763162 | I | 49.06 |
| CaD_SNP58 | CaLG_2 | 11423145 | I | 49.06 |
| CaD_SNP59 | CaLG_2 | 11423022 | I | 49.06 |
| CaD_SNP60 | scaffold04185 | 4924 | I | 49.06 |
| CaD_SNP61 | CaLG_7 | 1631890 | I | 49.06 |
| CaD_SNP62 | CaLG_4 | 5028184 | I | 49.06 |
| CaD_SNP63 | CaLG_7 | 1631320 | I | 49.06 |
| CaD_SNP64 | scaffold04185 | 7249 | I | 49.06 |
| CaD_SNP65 | CaLG_4 | 4878419 | I | 49.06 |
| CaD_SNP66 | CaLG_4 | 4936836 | I | 49.06 |
| CaD_SNP67 | scaffold00331 | 108727 | I | 49.06 |
| CaD_SNP68 | scaffold00331 | 116816 | I | 49.06 |
| CaD_SNP69 | scaffold00331 | 184677 | I | 49.06 |
| CaD_SNP70 | scaffold00331 | 123132 | I | 49.3 |
| CaD_SNP71 | scaffold00495 | 33630 | I | 49.53 |
| CaD_SNP72 | scaffold00331 | 47816 | I | 49.53 |
| CaD_SNP73 | scaffold00331 | 52242 | I | 49.53 |
| CaD_SNP74 | scaffold00906 | 87031 | I | 49.53 |
| CaD_SNP75 | scaffold00824 | 44982 | I | 49.53 |
| CaD_SNP76 | scaffold00824 | 42501 | I | 49.53 |
| CaD_SNP77 | scaffold00750 | 37856 | I | 49.53 |
| CaD_SNP78 | scaffold00660 | 92102 | I | 49.53 |
| CaD_SNP79 | scaffold04185 | 4807 | I | 49.53 |
| CaD_SNP80 | CaLG_2 | 11394153 | I | 49.53 |
| CaD_SNP81 | scaffold04102 | 11199 | I | 49.53 |
| CaD_SNP82 | scaffold00991 | 77662 | I | 49.53 |
| CaD_SNP83 | scaffold49682 | 128 | I | 53.36 |
| CaD_SNP84 | CaLG_4 | 5097092 | I | 57.29 |
| CaD_SNP85 | scaffold01034 | 25742 | I | 57.29 |
| CaD_SNP86 | scaffold01262 | 53659 | I | 57.29 |
| CaD_SNP87 | scaffold01438 | 24837 | I | 57.29 |
| CaD_SNP88 | CaLG_4 | 6325277 | I | 57.29 |
| CaD_SNP89 | scaffold01829 | 142943 | I | 57.29 |
| CaD_SNP90 | scaffold00723 | 27635 | I | 57.29 |
| CaD_SNP91 | scaffold00833 | 84456 | I | 57.29 |
| CaD_SNP92 | scaffold00883 | 52495 | I | 57.29 |

**Appendix H** Detailed information of SNPs used for QTL analysis of selected seed constituent traits (Continued).

| SNP ID | Chromosome number | Physical position (bp) | Linkage group | Map position (cM) |
|---|---|---|---|---|
| CaD_SNP93 | scaffold05171 | 13047 | I | 57.29 |
| CaD_SNP94 | scaffold05945 | 184695 | I | 57.29 |
| CaD_SNP95 | CaLG_1 | 10371032 | I | 57.29 |
| CaD_SNP96 | CaLG_1 | 10441613 | I | 57.29 |
| CaD_SNP97 | scaffold16742 | 53689 | I | 57.29 |
| CaD_SNP98 | CaLG_1 | 10441806 | I | 57.29 |
| CaD_SNP99 | scaffold03271 | 16062 | I | 57.29 |
| CaD_SNP100 | scaffold03271 | 11281 | I | 57.29 |
| CaD_SNP101 | scaffold03004 | 24459 | I | 57.29 |
| CaD_SNP102 | scaffold03258 | 4390 | I | 57.29 |
| CaD_SNP103 | scaffold02999 | 24593 | I | 57.29 |
| CaD_SNP104 | scaffold02278 | 258265 | I | 57.29 |
| CaD_SNP105 | scaffold02192 | 3958 | I | 57.29 |
| CaD_SNP106 | scaffold01149 | 231983 | I | 57.29 |
| CaD_SNP107 | scaffold01149 | 19503 | I | 57.29 |
| CaD_SNP108 | scaffold00723 | 43134 | I | 57.29 |
| CaD_SNP109 | scaffold00609 | 98741 | I | 57.29 |
| CaD_SNP110 | scaffold00609 | 116871 | I | 57.29 |
| CaD_SNP111 | scaffold00609 | 116688 | I | 57.29 |
| CaD_SNP112 | scaffold00609 | 111691 | I | 57.29 |
| CaD_SNP113 | scaffold00609 | 109266 | I | 57.29 |
| CaD_SNP114 | scaffold00609 | 109153 | I | 57.29 |
| CaD_SNP115 | scaffold00609 | 108803 | I | 57.29 |
| CaD_SNP116 | scaffold00609 | 107643 | I | 57.29 |
| CaD_SNP117 | scaffold00609 | 107480 | I | 57.29 |
| CaD_SNP118 | scaffold00450 | 42691 | I | 57.29 |
| CaD_SNP119 | scaffold00450 | 124388 | I | 57.29 |
| CaD_SNP120 | CaLG_4 | 5410884 | I | 57.29 |
| CaD_SNP121 | CaLG_4 | 5097206 | I | 57.29 |
| CaD_SNP122 | scaffold04570 | 6519 | I | 57.29 |
| CaD_SNP123 | scaffold04570 | 6408 | I | 57.29 |
| CaD_SNP124 | scaffold03792 | 19667 | I | 57.29 |
| CaD_SNP125 | CaLG_2 | 14163647 | I | 57.29 |
| CaD_SNP126 | scaffold03004 | 24760 | I | 57.29 |
| CaD_SNP127 | scaffold03004 | 24624 | I | 57.29 |
| CaD_SNP128 | scaffold03004 | 18460 | I | 57.29 |
| CaD_SNP129 | scaffold02835 | 207447 | I | 57.29 |
| CaD_SNP130 | scaffold02317 | 54463 | I | 57.29 |
| CaD_SNP131 | scaffold01989 | 15556 | I | 57.29 |
| CaD_SNP132 | scaffold01829 | 150289 | I | 57.29 |
| CaD_SNP133 | scaffold01194 | 78496 | I | 60.5 |
| CaD_SNP134 | scaffold00935 | 34654 | I | 76.26 |
| CaD_SNP135 | CaLG_4 | 8876149 | I | 76.51 |
| CaD_SNP136 | CaLG_4 | 8908491 | I | 76.51 |
| CaD_SNP137 | CaLG_4 | 8875920 | I | 76.51 |
| CaD_SNP138 | CaLG_4 | 8884501 | I | 76.51 |

**Appendix H** Detailed information of SNPs used for QTL analysis of selected seed constituent traits (Continued).

| SNP ID | Chromosome number | Physical position (bp) | Linkage group | Map position (cM) |
|---|---|---|---|---|
| CaD_SNP139 | CaLG_4 | 9224613 | I | 76.77 |
| CaD_SNP140 | CaLG_4 | 10237167 | I | 86.05 |
| CaD_SNP141 | CaLG_4 | 9856753 | I | 86.05 |
| CaD_SNP142 | CaLG_4 | 9855664 | I | 86.05 |
| CaD_SNP143 | CaLG_4 | 9795375 | I | 86.56 |
| CaD_SNP144 | CaLG_4 | 9858208 | I | 87.31 |
| CaD_SNP145 | CaLG_4 | 10249225 | I | 87.31 |
| CaD_SNP146 | CaLG_4 | 10376604 | I | 87.31 |
| CaD_SNP147 | CaLG_4 | 9851330 | I | 87.31 |
| CaD_SNP148 | scaffold11652 | 290 | I | 87.31 |
| CaD_SNP149 | scaffold01289 | 7943 | I | 91.3 |
| CaD_SNP150 | scaffold01289 | 42358 | I | 91.3 |
| CaD_SNP151 | scaffold01289 | 79287 | I | 91.54 |
| CaD_SNP152 | scaffold02437 | 24605 | I | 91.54 |
| CaD_SNP153 | CaLG_4 | 11089940 | I | 92.98 |
| CaD_SNP154 | scaffold00271 | 33776 | I | 93.7 |
| CaD_SNP155 | CaLG_4 | 11459017 | I | 93.93 |
| CaD_SNP156 | scaffold17943 | 416 | I | 93.93 |
| CaD_SNP157 | CaLG_4 | 11491028 | I | 93.93 |
| CaD_SNP158 | CaLG_4 | 11387866 | I | 93.93 |
| CaD_SNP159 | CaLG_4 | 11442514 | I | 93.93 |
| CaD_SNP160 | scaffold14718 | 783 | I | 93.93 |
| CaD_SNP161 | scaffold04888 | 12334 | I | 93.93 |
| CaD_SNP162 | scaffold04888 | 12127 | I | 93.93 |
| CaD_SNP163 | scaffold04888 | 10480 | I | 93.93 |
| CaD_SNP164 | scaffold00271 | 58847 | I | 94.4 |
| CaD_SNP165 | scaffold00271 | 79360 | I | 94.4 |
| CaD_SNP166 | CaLG_4 | 11585305 | I | 94.4 |
| CaD_SNP167 | CaLG_4 | 11570353 | I | 94.64 |
| CaD_SNP168 | CaLG_4 | 11543134 | I | 94.64 |
| CaD_SNP169 | CaLG_4 | 11540055 | I | 94.64 |
| CaD_SNP170 | CaLG_4 | 11572131 | I | 94.64 |
| CaD_SNP171 | scaffold00271 | 120137 | I | 94.64 |
| CaD_SNP172 | CaLG_4 | 11542258 | I | 94.64 |
| CaD_SNP173 | scaffold00271 | 120327 | I | 94.64 |
| CaD_SNP174 | CaLG_4 | 16303827 | I | 118.1 |
| CaD_SNP175 | CaLG_4 | 16219684 | I | 118.87 |
| CaD_SNP176 | CaLG_4 | 20580218 | I | 120.85 |
| CaD_SNP177 | scaffold06592 | 3618 | I | 121.09 |
| CaD_SNP178 | CaLG_4 | 20405127 | I | 121.09 |
| CaD_SNP179 | CaLG_4 | 20517968 | I | 121.09 |
| CaD_SNP180 | CaLG_4 | 20386593 | I | 121.09 |
| CaD_SNP181 | scaffold06592 | 4200 | I | 121.09 |
| CaD_SNP182 | CaLG_4 | 16900349 | I | 123.4 |
| CaD_SNP183 | CaLG_4 | 16900160 | I | 123.4 |
| CaD_SNP184 | CaLG_4 | 17026071 | I | 123.64 |

**Appendix H** Detailed information of SNPs used for QTL analysis of selected seed constituent traits (Continued).

| SNP ID | Chromosome number | Physical position (bp) | Linkage group | Map position (cM) |
|---|---|---|---|---|
| CaD_SNP185 | scaffold00263 | 160119 | I | 124.61 |
| CaD_SNP186 | scaffold01216 | 47600 | II | 0 |
| CaD_SNP187 | scaffold01216 | 47446 | II | 0 |
| CaD_SNP188 | scaffold01216 | 62521 | II | 0 |
| CaD_SNP189 | CaLG_7 | 8187265 | II | 5.94 |
| CaD_SNP190 | CaLG_6 | 10888348 | II | 18.87 |
| CaD_SNP191 | CaLG_6 | 5947664 | II | 24.73 |
| CaD_SNP192 | CaLG_6 | 4804535 | II | 34.25 |
| CaD_SNP193 | CaLG_6 | 7956395 | II | 39.21 |
| CaD_SNP194 | CaLG_6 | 7959943 | II | 39.21 |
| CaD_SNP195 | scaffold01291 | 29063 | II | 43.74 |
| CaD_SNP196 | CaLG_6 | 10095855 | II | 44.01 |
| CaD_SNP197 | CaLG_6 | 9379256 | II | 47.44 |
| CaD_SNP198 | CaLG_2 | 16064852 | II | 61.22 |
| CaD_SNP199 | scaffold00233 | 120461 | II | 64.5 |
| CaD_SNP200 | scaffold01394 | 1852 | II | 70.3 |
| CaD_SNP201 | scaffold13367 | 899 | II | 76.43 |
| CaD_SNP202 | scaffold02000 | 182 | II | 77.32 |
| CaD_SNP203 | scaffold31074 | 529 | II | 77.32 |
| CaD_SNP204 | CaLG_2 | 10439843 | II | 77.32 |
| CaD_SNP205 | scaffold60163 | 475 | II | 78.3 |
| CaD_SNP206 | CaLG_2 | 527633 | II | 89.45 |
| CaD_SNP207 | scaffold00806 | 74434 | II | 93.44 |
| CaD_SNP208 | CaLG_2 | 8013247 | II | 99.26 |
| CaD_SNP209 | CaLG_2 | 5550007 | II | 104.69 |
| CaD_SNP210 | scaffold00440 | 141645 | II | 105.65 |
| CaD_SNP211 | scaffold00440 | 161122 | II | 105.65 |
| CaD_SNP212 | scaffold00440 | 141021 | II | 105.65 |
| CaD_SNP213 | CaLG_7 | 2018132 | II | 113.07 |
| CaD_SNP214 | scaffold00837 | 92981 | II | 116.24 |
| CaD_SNP215 | CaLG_2 | 205617 | II | 117.62 |
| CaD_SNP216 | CaLG_1 | 461870 | III | 0 |
| CaD_SNP217 | CaLG_1 | 498862 | III | 0 |
| CaD_SNP218 | CaLG_3 | 17160394 | III | 9.89 |
| CaD_SNP219 | CaLG_3 | 15281781 | III | 33.44 |
| CaD_SNP220 | scaffold02872 | 23254 | III | 36.14 |
| CaD_SNP221 | scaffold07577 | 4158 | III | 40.95 |
| CaD_SNP222 | scaffold00640 | 101890 | III | 45.97 |
| CaD_SNP223 | CaLG_1 | 5061066 | III | 45.97 |
| CaD_SNP224 | CaLG_1 | 5094752 | III | 45.97 |
| CaD_SNP225 | CaLG_1 | 5004855 | III | 45.97 |
| CaD_SNP226 | scaffold01044 | 44938 | III | 45.97 |
| CaD_SNP227 | scaffold00640 | 12335 | III | 45.97 |
| CaD_SNP228 | scaffold00640 | 120278 | III | 45.97 |
| CaD_SNP229 | CaLG_1 | 5056328 | III | 45.97 |
| CaD_SNP230 | CaLG_1 | 5040663 | III | 45.97 |

**Appendix H** Detailed information of SNPs used for QTL analysis of selected seed constituent traits (Continued).

| SNP ID | Chromosome number | Physical position (bp) | Linkage group | Map position (cM) |
|---|---|---|---|---|
| CaD_SNP231 | CaLG_1 | 5056212 | III | 45.97 |
| CaD_SNP232 | CaLG_1 | 5807567 | III | 47.6 |
| CaD_SNP233 | scaffold01607 | 53631 | III | 50.34 |
| CaD_SNP234 | CaLG_1 | 6319012 | III | 52.73 |
| CaD_SNP235 | CaLG_1 | 6322174 | III | 52.73 |
| CaD_SNP236 | CaLG_1 | 6333418 | III | 52.73 |
| CaD_SNP237 | scaffold01172 | 61041 | III | 52.97 |
| CaD_SNP238 | CaLG_1 | 6313759 | III | 52.97 |
| CaD_SNP239 | scaffold00286 | 147990 | III | 55.52 |
| CaD_SNP240 | scaffold00286 | 164112 | III | 55.52 |
| CaD_SNP241 | scaffold02097 | 4507 | III | 55.77 |
| CaD_SNP242 | scaffold01670 | 22226 | III | 55.77 |
| CaD_SNP243 | scaffold00725 | 105354 | III | 55.77 |
| CaD_SNP244 | scaffold00509 | 12278 | III | 55.77 |
| CaD_SNP245 | scaffold00725 | 110465 | III | 55.77 |
| CaD_SNP246 | scaffold00853 | 21891 | III | 61.42 |
| CaD_SNP247 | scaffold15543 | 692 | III | 61.42 |
| CaD_SNP248 | scaffold01078 | 60476 | III | 61.42 |
| CaD_SNP249 | scaffold10990 | 36625 | III | 61.42 |
| CaD_SNP250 | CaLG_1 | 7606885 | III | 61.65 |
| CaD_SNP251 | scaffold01522 | 32033 | III | 61.65 |
| CaD_SNP252 | CaLG_1 | 7632307 | III | 61.65 |
| CaD_SNP253 | scaffold00798 | 26164 | III | 61.65 |
| CaD_SNP254 | scaffold02259 | 28518 | III | 61.65 |
| CaD_SNP255 | scaffold02259 | 28398 | III | 61.65 |
| CaD_SNP256 | scaffold09892 | 56292 | III | 61.65 |
| CaD_SNP257 | scaffold04196 | 52526 | III | 61.65 |
| CaD_SNP258 | scaffold04196 | 49463 | III | 61.65 |
| CaD_SNP259 | scaffold04196 | 36997 | III | 61.65 |
| CaD_SNP260 | scaffold04196 | 28086 | III | 61.65 |
| CaD_SNP261 | CaLG_4 | 5746110 | III | 61.65 |
| CaD_SNP262 | CaLG_1 | 9014610 | III | 68.95 |
| CaD_SNP263 | CaLG_1 | 8871945 | III | 70.4 |
| CaD_SNP264 | scaffold03415 | 27496 | III | 73.98 |
| CaD_SNP265 | scaffold00205 | 89221 | III | 75 |
| CaD_SNP266 | scaffold00360 | 175256 | III | 75.71 |
| CaD_SNP267 | scaffold02401 | 12927 | III | 87.08 |
| CaD_SNP268 | scaffold02085 | 24455 | III | 87.32 |
| CaD_SNP269 | CaLG_1 | 11569811 | III | 91.83 |
| CaD_SNP270 | scaffold04082 | 2195 | III | 100.61 |
| CaD_SNP271 | CaLG_4 | 4053260 | IV | 0 |
| CaD_SNP272 | CaLG_4 | 4053361 | IV | 0 |
| CaD_SNP273 | CaLG_4 | 4018936 | IV | 0.25 |
| CaD_SNP274 | CaLG_4 | 4018698 | IV | 0.25 |
| CaD_SNP275 | scaffold06248 | 6259 | IV | 0.25 |
| CaD_SNP276 | CaLG_4 | 4018828 | IV | 0.25 |

**Appendix H** Detailed information of SNPs used for QTL analysis of selected seed constituent traits (Continued).

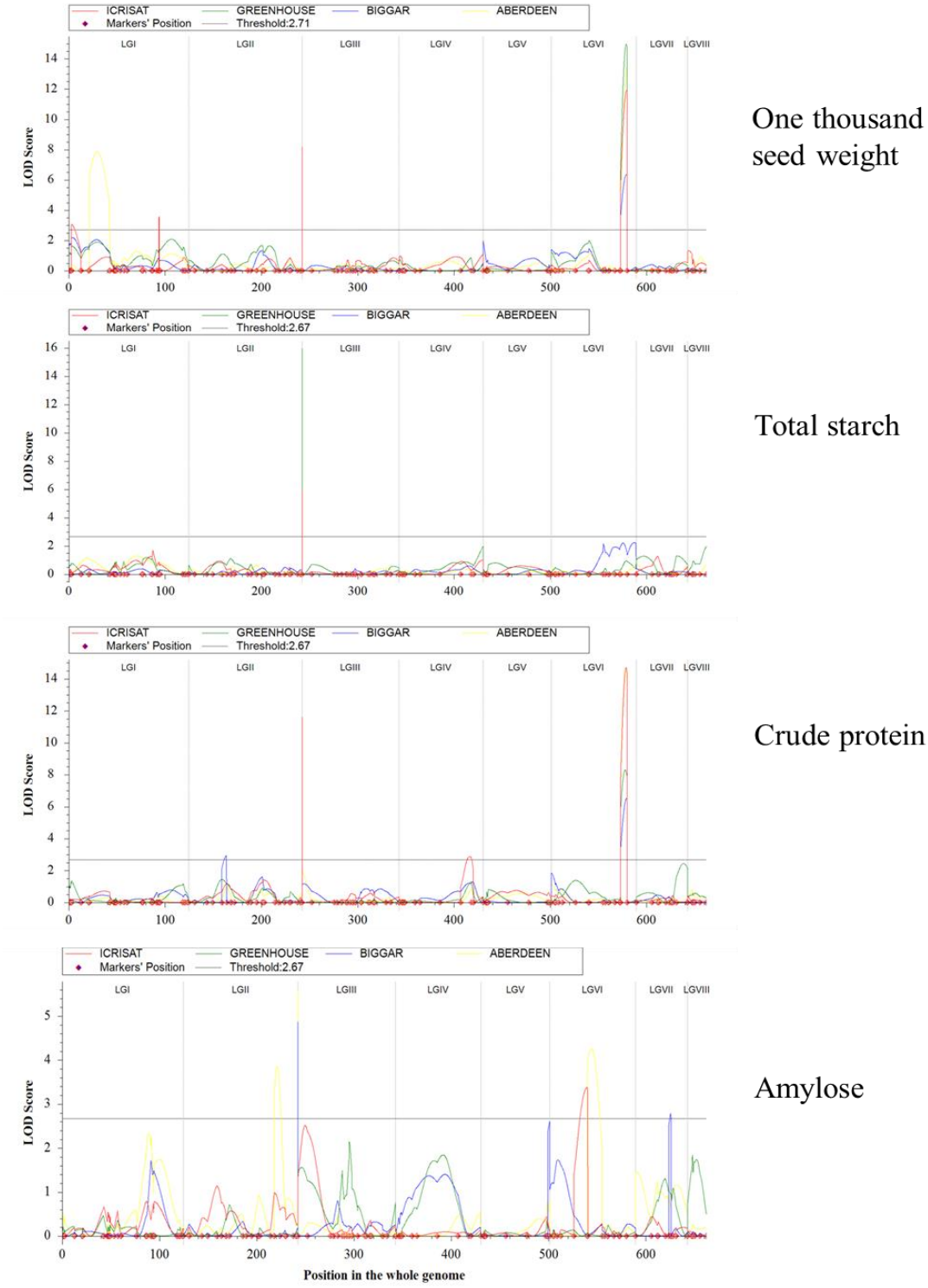
| SNP ID | Chromosome number | Physical position (bp) | Linkage group | Map position (cM) |
|---|---|---|---|---|
| CaD_SNP277 | scaffold01326 | 18720 | IV | 0.5 |
| CaD_SNP278 | scaffold01326 | 30430 | IV | 0.73 |
| CaD_SNP279 | CaLG_6 | 816771 | IV | 3.28 |
| CaD_SNP280 | CaLG_6 | 785583 | IV | 3.77 |
| CaD_SNP281 | scaffold01478 | 53238 | IV | 4.24 |
| CaD_SNP282 | scaffold01478 | 58213 | IV | 4.24 |
| CaD_SNP283 | scaffold01478 | 58950 | IV | 4.24 |
| CaD_SNP284 | scaffold01478 | 64875 | IV | 4.24 |
| CaD_SNP285 | scaffold01478 | 59052 | IV | 4.24 |
| CaD_SNP286 | scaffold00728 | 15128 | IV | 5.18 |
| CaD_SNP287 | scaffold00728 | 15500 | IV | 5.18 |
| CaD_SNP288 | CaLG_2 | 8073314 | IV | 5.18 |
| CaD_SNP289 | scaffold09991 | 1459 | IV | 5.18 |
| CaD_SNP290 | scaffold00696 | 123800 | IV | 5.41 |
| CaD_SNP291 | scaffold00696 | 114930 | IV | 5.41 |
| CaD_SNP292 | scaffold00696 | 104936 | IV | 5.41 |
| CaD_SNP293 | scaffold02114 | 38150 | IV | 5.88 |
| CaD_SNP294 | scaffold02749 | 34073 | IV | 6.58 |
| CaD_SNP295 | CaLG_2 | 7697803 | IV | 6.58 |
| CaD_SNP296 | CaLG_2 | 6437145 | IV | 16.71 |
| CaD_SNP297 | scaffold04899 | 1851 | IV | 21.96 |
| CaD_SNP298 | scaffold65057 | 431 | IV | 42.74 |
| CaD_SNP299 | CaLG_2 | 1048614 | IV | 64.15 |
| CaD_SNP300 | scaffold02625 | 3288 | IV | 75.12 |
| CaD_SNP301 | scaffold00207 | 3822 | IV | 77.44 |
| CaD_SNP302 | CaLG_6 | 4231750 | IV | 87.45 |
| CaD_SNP303 | scaffold00055 | 325897 | V | 0 |
| CaD_SNP304 | scaffold00917 | 7110 | V | 2.8 |
| CaD_SNP305 | scaffold00877 | 213456 | V | 3.45 |
| CaD_SNP306 | CaLG_7 | 2541403 | V | 3.45 |
| CaD_SNP307 | scaffold04114 | 835 | V | 3.45 |
| CaD_SNP308 | scaffold17669 | 1399 | V | 3.71 |
| CaD_SNP309 | CaLG_7 | 3675434 | V | 3.96 |
| CaD_SNP310 | scaffold03119 | 2156 | V | 4.66 |
| CaD_SNP311 | CaLG_7 | 5138627 | V | 5.39 |
| CaD_SNP312 | CaLG_7 | 5137821 | V | 5.39 |
| CaD_SNP313 | scaffold00407 | 21052 | V | 25.61 |
| CaD_SNP314 | CaLG_8 | 6392071 | V | 47.56 |
| CaD_SNP315 | CaLG_8 | 4126353 | V | 66.58 |
| CaD_SNP316 | CaLG_8 | 3656251 | V | 68.28 |
| CaD_SNP317 | CaLG_8 | 3810985 | V | 68.28 |
| CaD_SNP318 | CaLG_8 | 3804401 | V | 68.28 |
| CaD_SNP319 | CaLG_8 | 3718115 | V | 68.28 |
| CaD_SNP320 | CaLG_8 | 3680544 | V | 68.56 |
| CaD_SNP321 | CaLG_8 | 3659352 | V | 69.04 |
| CaD_SNP322 | CaLG_8 | 3789039 | V | 69.04 |

**Appendix H** Detailed information of SNPs used for QTL analysis of selected seed constituent traits (Continued).

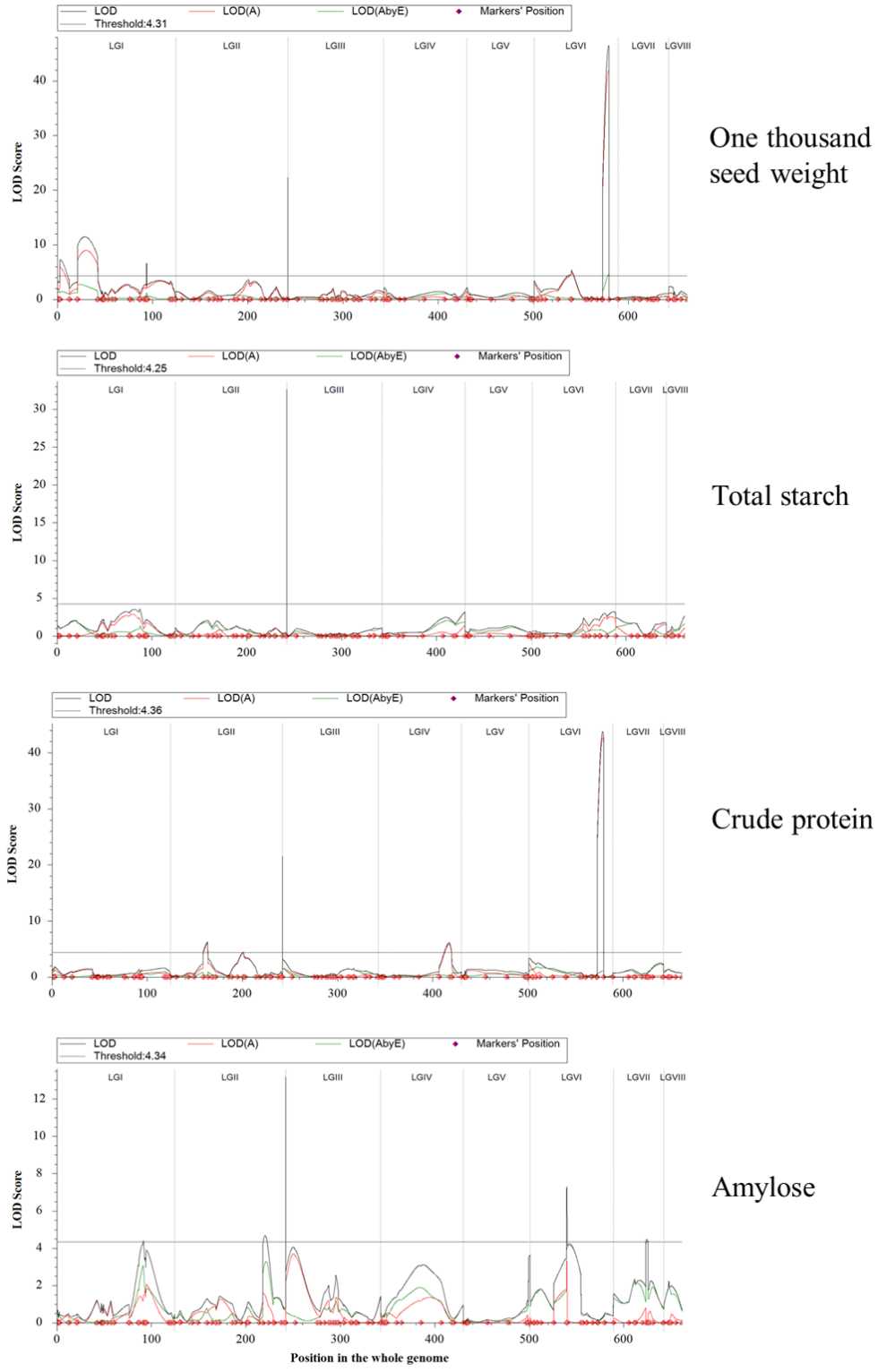
| SNP ID | Chromosome number | Physical position (bp) | Linkage group | Map position (cM) |
|---|---|---|---|---|
| CaD_SNP323 | CaLG_8 | 3672982 | V | 70.94 |
| CaD_SNP324 | CaLG_3 | 14172702 | VI | 0 |
| CaD_SNP325 | scaffold110072 | 306 | VI | 3.72 |
| CaD_SNP326 | scaffold00383 | 55779 | VI | 4.28 |
| CaD_SNP327 | scaffold00252 | 41046 | VI | 4.53 |
| CaD_SNP328 | scaffold02136 | 19060 | VI | 7.46 |
| CaD_SNP329 | CaLG_3 | 11599239 | VI | 11.31 |
| CaD_SNP330 | CaLG_3 | 11115658 | VI | 24.81 |
| CaD_SNP331 | CaLG_3 | 8828383 | VI | 38.68 |
| CaD_SNP332 | CaLG_3 | 8974953 | VI | 39.17 |
| CaD_SNP333 | CaLG_3 | 6758389 | VI | 53.81 |
| CaD_SNP334 | CaLG_3 | 6799092 | VI | 53.81 |
| CaD_SNP335 | CaLG_3 | 6812090 | VI | 53.81 |
| CaD_SNP336 | CaLG_3 | 6812195 | VI | 53.81 |
| CaD_SNP337 | CaLG_3 | 6758514 | VI | 53.81 |
| CaD_SNP338 | scaffold07031 | 2083 | VI | 53.81 |
| CaD_SNP339 | CaLG_3 | 6723814 | VI | 53.81 |
| CaD_SNP340 | CaLG_3 | 6738992 | VI | 53.81 |
| CaD_SNP341 | CaLG_3 | 6720816 | VI | 53.81 |
| CaD_SNP342 | CaLG_3 | 6544127 | VI | 53.81 |
| CaD_SNP343 | CaLG_3 | 6544021 | VI | 53.81 |
| CaD_SNP344 | CaLG_3 | 6543089 | VI | 53.81 |
| CaD_SNP345 | CaLG_3 | 6436024 | VI | 53.81 |
| CaD_SNP346 | CaLG_3 | 6435914 | VI | 53.81 |
| CaD_SNP347 | CaLG_3 | 6434644 | VI | 53.81 |
| CaD_SNP348 | CaLG_3 | 6390861 | VI | 53.81 |
| CaD_SNP349 | CaLG_3 | 6547513 | VI | 53.81 |
| CaD_SNP350 | CaLG_3 | 6547617 | VI | 53.81 |
| CaD_SNP351 | CaLG_3 | 6593914 | VI | 53.81 |
| CaD_SNP352 | scaffold00198 | 221315 | VI | 56.13 |
| CaD_SNP353 | scaffold02451 | 2535 | VI | 59.96 |
| CaD_SNP354 | CaLG_3 | 4788995 | VI | 65.79 |
| CaD_SNP355 | CaLG_3 | 3973381 | VI | 71.15 |
| CaD_SNP356 | CaLG_3 | 4039042 | VI | 71.15 |
| CaD_SNP357 | CaLG_3 | 4086443 | VI | 71.15 |
| CaD_SNP358 | CaLG_3 | 4100572 | VI | 71.15 |
| CaD_SNP359 | CaLG_3 | 4019445 | VI | 71.15 |
| CaD_SNP360 | CaLG_3 | 4211455 | VI | 71.15 |
| CaD_SNP361 | CaLG_3 | 3967388 | VI | 71.15 |
| CaD_SNP362 | CaLG_3 | 4182356 | VI | 71.15 |
| CaD_SNP363 | CaLG_3 | 4100725 | VI | 71.15 |
| CaD_SNP364 | CaLG_3 | 4197306 | VI | 71.15 |
| CaD_SNP365 | CaLG_3 | 4197482 | VI | 71.15 |
| CaD_SNP366 | CaLG_3 | 4356981 | VI | 71.38 |
| CaD_SNP367 | CaLG_3 | 4415704 | VI | 71.62 |
| CaD_SNP368 | CaLG_3 | 4261374 | VI | 71.87 |

**Appendix H** Detailed information of SNPs used for QTL analysis of selected seed constituent traits (Continued).

| SNP ID | Chromosome number | Physical position (bp) | Linkage group | Map position (cM) |
|---|---|---|---|---|
| CaD_SNP369 | CaLG_3 | 4314473 | VI | 71.87 |
| CaD_SNP370 | CaLG_3 | 4347515 | VI | 71.87 |
| CaD_SNP371 | CaLG_3 | 4389997 | VI | 71.87 |
| CaD_SNP372 | CaLG_3 | 3512869 | VI | 78.58 |
| CaD_SNP373 | CaLG_3 | 3493826 | VI | 78.58 |
| CaD_SNP374 | CaLG_3 | 3488455 | VI | 78.58 |
| CaD_SNP375 | CaLG_3 | 3495509 | VI | 78.58 |
| CaD_SNP376 | CaLG_3 | 3611183 | VI | 78.81 |
| CaD_SNP377 | CaLG_3 | 3609578 | VI | 78.81 |
| CaD_SNP378 | scaffold01120 | 90149 | VI | 88.01 |
| CaD_SNP379 | CaLG_5 | 9907069 | VII | 0 |
| CaD_SNP380 | scaffold01290 | 25651 | VII | 16.45 |
| CaD_SNP381 | scaffold00748 | 72149 | VII | 22.62 |
| CaD_SNP382 | CaLG_5 | 6458466 | VII | 23.47 |
| CaD_SNP383 | CaLG_5 | 11987686 | VII | 30.71 |
| CaD_SNP384 | scaffold01444 | 20342 | VII | 34.47 |
| CaD_SNP385 | scaffold00113 | 65093 | VII | 36.42 |
| CaD_SNP386 | scaffold00113 | 140380 | VII | 36.69 |
| CaD_SNP387 | CaLG_5 | 13191923 | VII | 36.69 |
| CaD_SNP388 | CaLG_5 | 13192535 | VII | 36.69 |
| CaD_SNP389 | scaffold00113 | 292224 | VII | 36.69 |
| CaD_SNP390 | scaffold00113 | 206141 | VII | 36.69 |
| CaD_SNP391 | scaffold00113 | 154538 | VII | 36.69 |
| CaD_SNP392 | scaffold00978 | 78175 | VII | 37.55 |
| CaD_SNP393 | scaffold28189 | 780 | VII | 38.72 |
| CaD_SNP394 | scaffold00631 | 189756 | VII | 41.75 |
| CaD_SNP395 | scaffold06790 | 7969 | VII | 53.25 |
| CaD_SNP396 | scaffold00844 | 100250 | VII | 53.55 |
| CaD_SNP397 | scaffold00601 | 5841 | VIII | 0 |
| CaD_SNP398 | CaLG_3 | 22992745 | VIII | 4.58 |
| CaD_SNP399 | scaffold01937 | 8146 | VIII | 4.58 |
| CaD_SNP400 | scaffold01937 | 55399 | VIII | 4.58 |
| CaD_SNP401 | scaffold01937 | 10395 | VIII | 4.58 |
| CaD_SNP402 | CaLG_3 | 22992410 | VIII | 4.58 |
| CaD_SNP403 | CaLG_3 | 22985057 | VIII | 4.58 |
| CaD_SNP404 | CaLG_3 | 22984947 | VIII | 4.58 |
| CaD_SNP405 | CaLG_3 | 22980522 | VIII | 4.58 |
| CaD_SNP406 | CaLG_3 | 22973073 | VIII | 4.58 |
| CaD_SNP407 | CaLG_7 | 1558737 | VIII | 5.17 |
| CaD_SNP408 | scaffold02312 | 3186 | VIII | 5.17 |
| CaD_SNP409 | scaffold00257 | 53147 | VIII | 5.17 |
| CaD_SNP410 | CaLG_7 | 1541646 | VIII | 5.17 |
| CaD_SNP411 | CaLG_7 | 1566076 | VIII | 5.17 |
| CaD_SNP412 | scaffold01937 | 8281 | VIII | 5.76 |
| CaD_SNP413 | CaLG_7 | 1566197 | VIII | 7.61 |
| CaD_SNP414 | scaffold00898 | 105939 | VIII | 12.84 |
| CaD_SNP415 | scaffold00812 | 146 | VIII | 19.43 |




**Appendix I** LOD profile of QTLs identified on linkage groups of I – VIII for selected seed constituent traits in different environments.
ICRISAT (F 2011), greenhouse (G 2012), Biggar (F 2012) and Aberdeen (F 2013).




**Appendix J** LOD profile of QTLs by environment interaction for selected seed constituent traits.
The black line represents the total LOD caused by both the additive effect (A) and the additive effect by environment interaction (AbyE). The red line represents LOD caused by the additive effect, whereas the green line stands for LOD caused by the additive effect by environment interaction.

**Appendix K** Epistatic QTLs identified for TSW in multi-environmental test (MET).

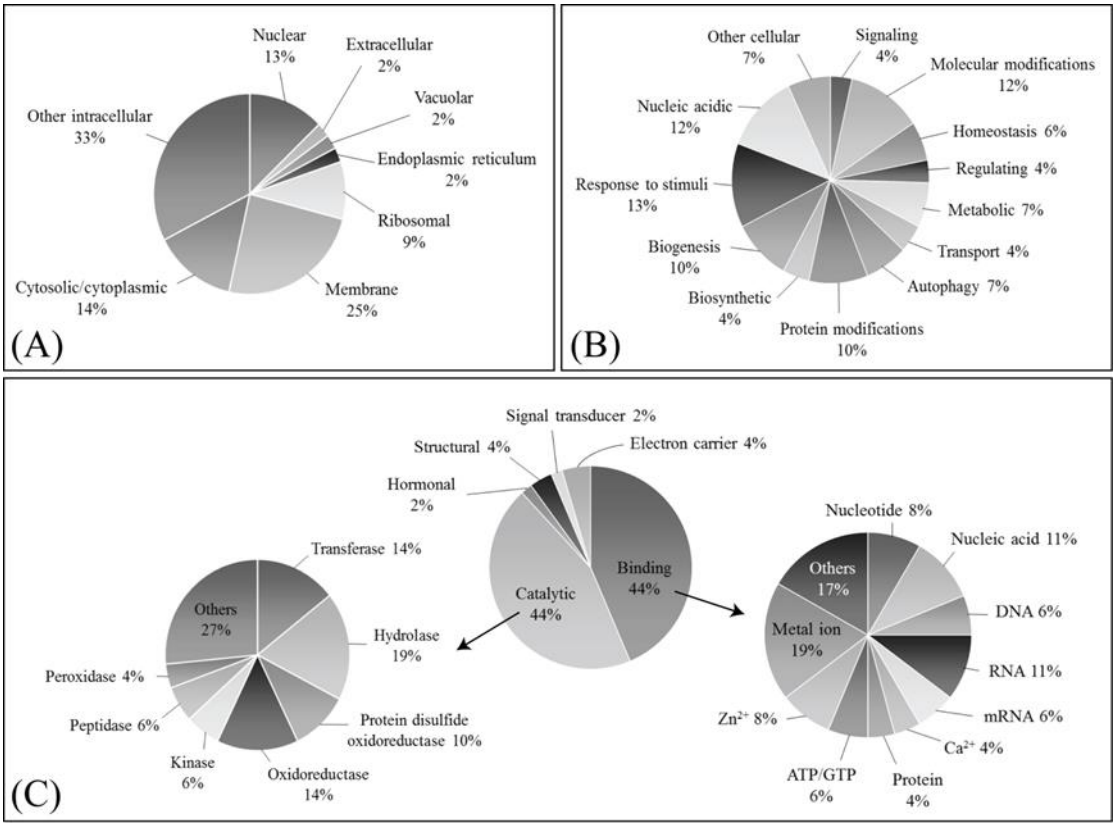
| Trait | LG 1 | Position 1 (cM)[a] | Left Marker 1[b] | Right Marker 1[b] | LG 2 | Position 2 (cM)[a] | Left Marker 2[b] | Right Marker 2[b] | LOD | LOD (AA)[c] | LOD (AAbyE)[d] | PVE (%) | PVE (AA) (%)[c] | PVE (AAbyE) (%)[d] | Add 1[e] | Add 2[e] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | 47.0 | CaD_SNP30 | CaD_SNP31 | I | 53.0 | CaD_SNP82 | CaD_SNP83 | 8.10 | 6.11 | 1.99 | 0.60 | 0.60 | 0.00 | 19.99 | -16.61 |
| | II | 61.0 | CaD_SNP197 | CaD_SNP198 | II | 70.0 | CaD_SNP199 | CaD_SNP200 | 6.38 | 5.88 | 0.50 | 2.13 | 1.86 | 0.27 | -1.03 | 3.41 |
| | I | 75.0 | CaD_SNP133 | CaD_SNP134 | III | 5.0 | CaD_SNP217 | CaD_SNP218 | 6.96 | 5.53 | 1.43 | 2.03 | 1.78 | 0.25 | 5.53 | -1.02 |
| | IV | 0.0 | CaD_SNP272 | CaD_SNP273 | IV | 18.0 | CaD_SNP296 | CaD_SNP297 | 7.90 | 6.76 | 1.13 | 2.33 | 1.97 | 0.36 | 3.90 | -2.06 |
| | III | 50.0 | CaD_SNP232 | CaD_SNP233 | IV | 75.0 | CaD_SNP299 | CaD_SNP300 | 7.26 | 5.31 | 1.95 | 2.68 | 1.83 | 0.85 | -2.38 | 3.20 |
| | III | 34.0 | CaD_SNP219 | CaD_SNP220 | V | 7.0 | CaD_SNP312 | CaD_SNP313 | 6.03 | 5.07 | 0.96 | 2.23 | 1.75 | 0.48 | -1.01 | 1.67 |
| TSW | V | 6.0 | CaD_SNP312 | CaD_SNP313 | V | 59.0 | CaD_SNP314 | CaD_SNP315 | 5.91 | 5.63 | 0.28 | 2.32 | 2.17 | 0.16 | 1.01 | -1.87 |
| | IV | 6.0 | CaD_SNP293 | CaD_SNP294 | VI | 4.0 | CaD_SNP325 | CaD_SNP326 | 6.19 | 5.90 | 0.29 | 2.11 | 2.01 | 0.10 | 2.45 | -2.65 |
| | II | 99.0 | CaD_SNP207 | CaD_SNP208 | VI | 10.0 | CaD_SNP328 | CaD_SNP329 | 5.93 | 5.31 | 0.62 | 2.12 | 1.84 | 0.28 | -1.65 | -3.46 |
| | III | 55.0 | CaD_SNP238 | CaD_SNP239 | VI | 54.0 | CaD_SNP351 | CaD_SNP352 | 8.15 | 7.44 | 0.71 | 2.91 | 2.56 | 0.34 | -2.86 | 2.08 |
| | I | 33.0 | CaD_SNP26 | CaD_SNP27 | VI | 72.0 | CaD_SNP371 | CaD_SNP372 | 10.87 | 8.91 | 1.96 | 4.74 | 3.73 | 1.01 | 10.45 | 14.52 |
| | IV | 52.0 | CaD_SNP298 | CaD_SNP299 | VII | 0.0 | CaD_SNP379 | CaD_SNP380 | 6.86 | 6.13 | 0.73 | 2.67 | 2.26 | 0.40 | -0.99 | 0.85 |
| | IV | 87.0 | CaD_SNP301 | CaD_SNP302 | VIII | 19.0 | CaD_SNP414 | CaD_SNP415 | 7.41 | 6.57 | 0.84 | 2.68 | 2.25 | 0.43 | -3.59 | 1.57 |

a, the position of the LOD peak.
b, the markers flanking the position of the LOD peak.
c, LOD or PVE caused by the additive × additive effect.
d, LOD or PVE caused by the interaction of the additive × additive effect with the environment.
e, positive values indicate alleles are donated by ICC 995, whereas negative values indicate alleles are donated by ICC 5912.

**Appendix L** Epistatic QTLs identified for total starch, protein and amylose in multi-environmental test (MET).

| Trait | LG 1 | Position 1 (cM)[a] | Left Marker 1[b] | Right Marker 1[b] | LG 2 | Position 2 (cM)[a] | Left Marker 2[b] | Right Marker 2[b] | LOD | LOD (AA)[c] | LOD (AAbyE)[d] | PVE (%) | PVE (AA) (%)[c] | PVE (AAbyE) (%)[d] | Add 1[e] | Add 2[e] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total starch | I | 119.0 | CaD_SNP175 | CaD_SNP176 | IV | 86.0 | CaD_SNP301 | CaD_SNP302 | 7.01 | 5.62 | 1.39 | 2.49 | 2.07 | 0.42 | 0.00 | -0.36 |
| | V | 26.0 | CaD_SNP313 | CaD_SNP314 | VI | 54.0 | CaD_SNP351 | CaD_SNP352 | 6.04 | 3.71 | 2.33 | 2.40 | 1.39 | 1.01 | 0.25 | 0.38 |
| | II | 71.0 | CaD_SNP200 | CaD_SNP201 | VIII | 15.0 | CaD_SNP414 | CaD_SNP415 | 7.16 | 4.92 | 2.24 | 2.67 | 1.79 | 0.88 | 0.12 | 0.23 |
| Protein | I | 21.0 | CaD_SNP23 | CaD_SNP24 | III | 52.0 | CaD_SNP233 | CaD_SNP234 | 5.83 | 5.54 | 0.29 | 2.06 | 1.97 | 0.09 | -0.20 | -0.05 |
| | II | 106.0 | CaD_SNP212 | CaD_SNP213 | III | 53.0 | CaD_SNP238 | CaD_SNP239 | 5.15 | 4.66 | 0.49 | 1.85 | 1.68 | 0.17 | 0.02 | -0.09 |
| | IV | 1.0 | CaD_SNP278 | CaD_SNP279 | IV | 17.0 | CaD_SNP296 | CaD_SNP297 | 12.55 | 12.52 | 0.03 | 4.42 | 4.42 | 0.00 | -0.02 | -0.02 |
| | III | 51.0 | CaD_SNP233 | CaD_SNP234 | IV | 75.0 | CaD_SNP299 | CaD_SNP300 | 6.52 | 6.28 | 0.25 | 2.28 | 2.21 | 0.07 | -0.05 | -0.41 |
| | IV | 60.0 | CaD_SNP298 | CaD_SNP299 | VI | 12.0 | CaD_SNP329 | CaD_SNP330 | 5.99 | 5.11 | 0.88 | 2.13 | 1.85 | 0.28 | -0.07 | 0.08 |
| | II | 71.0 | CaD_SNP200 | CaD_SNP201 | VI | 51.0 | CaD_SNP332 | CaD_SNP333 | 8.61 | 8.37 | 0.24 | 3.00 | 2.92 | 0.07 | -0.25 | -0.15 |
| | V | 26.0 | CaD_SNP313 | CaD_SNP314 | VI | 54.0 | CaD_SNP351 | CaD_SNP352 | 5.37 | 5.29 | 0.07 | 1.94 | 1.91 | 0.03 | -0.14 | -0.11 |
| | I | 22.0 | CaD_SNP26 | CaD_SNP27 | VI | 71.0 | CaD_SNP354 | CaD_SNP355 | 9.25 | 9.20 | 0.05 | 3.36 | 3.35 | 0.02 | -0.23 | -0.05 |
| | IV | 65.0 | CaD_SNP299 | CaD_SNP300 | VII | 39.0 | CaD_SNP393 | CaD_SNP394 | 5.56 | 5.37 | 0.19 | 2.05 | 1.99 | 0.06 | -0.18 | -0.01 |
| | V | 47.0 | CaD_SNP313 | CaD_SNP314 | VII | 39.0 | CaD_SNP393 | CaD_SNP394 | 5.15 | 4.73 | 0.41 | 1.88 | 1.71 | 0.17 | -0.08 | -0.06 |
| | I | 124.0 | CaD_SNP184 | CaD_SNP185 | VIII | 7.0 | CaD_SNP412 | CaD_SNP413 | 10.22 | 10.04 | 0.17 | 3.75 | 3.70 | 0.05 | 0.00 | 0.04 |
| | IV | 87.0 | CaD_SNP301 | CaD_SNP302 | VIII | 19.0 | CaD_SNP414 | CaD_SNP415 | 5.26 | 4.53 | 0.73 | 1.83 | 1.63 | 0.21 | 0.07 | 0.01 |
| Amylose | II | 15.0 | CaD_SNP189 | CaD_SNP190 | II | 71.0 | CaD_SNP200 | CaD_SNP201 | 6.48 | 4.39 | 2.09 | 2.80 | 1.68 | 1.12 | -0.05 | -0.02 |
| | I | 0.0 | CaD_SNP1 | CaD_SNP2 | III | 100.0 | CaD_SNP269 | CaD_SNP270 | 5.70 | 3.11 | 2.60 | 2.24 | 1.39 | 0.85 | -0.05 | -0.03 |
| | II | 18.0 | CaD_SNP189 | CaD_SNP190 | IV | 3.0 | CaD_SNP278 | CaD_SNP279 | 5.24 | 2.37 | 2.87 | 2.70 | 1.02 | 1.68 | -0.10 | -0.02 |
| | IV | 4.0 | CaD_SNP280 | CaD_SNP281 | IV | 7.0 | CaD_SNP295 | CaD_SNP296 | 5.00 | 2.54 | 2.46 | 0.89 | 0.55 | 0.34 | -0.22 | 0.29 |
| | I | 118.0 | CaD_SNP173 | CaD_SNP174 | V | 2.0 | CaD_SNP303 | CaD_SNP304 | 5.11 | 2.44 | 2.67 | 2.17 | 1.10 | 1.07 | 0.01 | -0.01 |
| | V | 69.0 | CaD_SNP320 | CaD_SNP321 | VI | 10.0 | CaD_SNP328 | CaD_SNP329 | 5.57 | 2.87 | 2.70 | 2.51 | 1.29 | 1.23 | 0.07 | -0.02 |
| | III | 17.0 | CaD_SNP218 | CaD_SNP219 | VII | 0.0 | CaD_SNP379 | CaD_SNP380 | 5.17 | 1.89 | 3.28 | 1.46 | 0.63 | 0.83 | -0.22 | -0.08 |

a, the position of the LOD peak.
b, the markers flanking the position of the LOD peak.
c, LOD or PVE caused by the additive × additive effect.
d, LOD or PVE caused by the interaction of the additive × additive effect with the environment.
e, positive values indicate alleles are donated by ICC 995, whereas negative values indicate alleles are donated by ICC 5912.




**Appendix M** Functional annotation, by gene ontology, of putative genes present in quantitative trait loci on chromosome 3 of desi type chickpea.
(A) cellular component, (B) biological process, and (C) molecular function. The QTL is flanked by CaD_SNP371 and CaD_SNP372 markers.

**Appendix N** Summary of values for selected seed constituent traits in desi chickpea.

| Genotype No. | TSW (g) | | Total starch (%) | | Amylose (%) | | Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 1 | 168.1 | 27.1 | 37.7 | 2.6 | 32.3 | 3.0 | 18.7 | 0.1 |
| 2 | 198.3 | 23.0 | 38.9 | 6.9 | 32.6 | 2.8 | 17.0 | 1.6 |
| 3 | 317.3 | 16.6 | 42.4 | 7.3 | 32.8 | 3.0 | 18.7 | 1.5 |
| 4 | 178.5 | 25.1 | 36.0 | 2.9 | 32.6 | 3.9 | 20.5 | 2.0 |
| 5 | 184.3 | 30.1 | 36.0 | 2.9 | 32.7 | 2.6 | 20.9 | 0.4 |
| 6 | 174.4 | 16.6 | 37.1 | 5.1 | 33.4 | 2.0 | 18.7 | 1.0 |
| 7 | 181.4 | 35.3 | 36.8 | 3.7 | 33.2 | 3.9 | 18.3 | 1.4 |
| 8 | 225.0 | 26.6 | 41.4 | 3.6 | 31.9 | 3.4 | 18.6 | 1.0 |
| 9 | 114.6 | 9.2 | 36.8 | 2.0 | 32.2 | 2.5 | 25.6 | 3.7 |
| 10 | 199.1 | 26.8 | 42.9 | 3.6 | 31.9 | 3.2 | 18.0 | 2.3 |
| 11 | 270.1 | 48.6 | 41.4 | 3.9 | 31.4 | 3.3 | 18.5 | 1.2 |
| 12 | 138.5 | 41.0 | 39.0 | 2.1 | 32.3 | 3.3 | 20.1 | 0.2 |
| 13 | 218.1 | 44.4 | 40.7 | 2.3 | 31.6 | 4.3 | 19.9 | 0.9 |
| 14 | 143.8 | 40.6 | 36.5 | 2.9 | 33.0 | 2.4 | 23.9 | 4.0 |
| 15 | 126.5 | 31.6 | 35.8 | 1.6 | 34.2 | 2.8 | 23.1 | 5.2 |
| 16 | 137.5 | 27.0 | 37.8 | 2.1 | 33.8 | 2.8 | 19.9 | 3.1 |
| 17 | 140.4 | 40.1 | 32.4 | 3.4 | 33.1 | 2.8 | 21.7 | 4.2 |
| 18 | 146.9 | 29.1 | 36.5 | 3.1 | 33.0 | 2.7 | 22.1 | 3.4 |
| 19 | 197.7 | 35.3 | 38.3 | 2.2 | 33.6 | 4.3 | 20.9 | 3.6 |
| 20 | 206.2 | 27.9 | 35.7 | 1.2 | 31.3 | 1.0 | 20.9 | 3.4 |
| 21 | 140.3 | 35.9 | 37.2 | 3.4 | 32.6 | 2.7 | 23.2 | 4.7 |
| 22 | 155.9 | 32.3 | 37.9 | 1.7 | 33.1 | 2.5 | 23.4 | 3.5 |
| 23 | 139.5 | 34.9 | 36.6 | 3.3 | 32.6 | 2.6 | 22.1 | 3.0 |
| 24 | 146.6 | 42.3 | 36.8 | 1.0 | 33.1 | 2.7 | 22.6 | 4.5 |
| 25 | 130.8 | 26.2 | 34.9 | 3.0 | 32.1 | 2.0 | 22.4 | 3.0 |
| 26 | 140.5 | 44.7 | 38.4 | 1.7 | 33.4 | 2.8 | 21.8 | 2.2 |
| 27 | 154.2 | 18.8 | 37.5 | 2.2 | 32.6 | 2.7 | 21.5 | 2.7 |
| 28 | 128.0 | 21.8 | 37.9 | 3.3 | 32.1 | 1.8 | 22.0 | 3.4 |
| 29 | 137.1 | 25.8 | 37.5 | 2.6 | 32.3 | 1.8 | 22.7 | 3.1 |
| 30 | 148.6 | 39.5 | 37.4 | 3.1 | 33.1 | 2.9 | 22.0 | 2.9 |
| 31 | 120.6 | 25.2 | 35.3 | 1.1 | 31.3 | 2.8 | 19.9 | 2.9 |
| 32 | 135.5 | 41.8 | 38.6 | 3.4 | 33.5 | 2.7 | 23.8 | 4.3 |
| 33 | 175.6 | 26.5 | 36.9 | 1.6 | 33.5 | 1.8 | 21.2 | 2.6 |
| 34 | 127.2 | 30.2 | 35.4 | 4.0 | 33.2 | 3.3 | 21.7 | 3.9 |
| 35 | 185.6 | 26.9 | 34.0 | 1.8 | 34.0 | 2.7 | 22.6 | 3.0 |
| 36 | 165.1 | 34.2 | 37.2 | 1.5 | 31.6 | 1.4 | 21.5 | 4.0 |
| 37 | 115.8 | 9.2 | 38.0 | 1.0 | 32.9 | 1.9 | 27.1 | 2.0 |
| 38 | 239.0 | 36.4 | 40.5 | 1.8 | 33.3 | 2.1 | 20.5 | 3.0 |
| 39 | 129.0 | 27.5 | 37.4 | 2.0 | 32.7 | 2.3 | 20.5 | 4.1 |
| 40 | 181.9 | 32.4 | 36.4 | 1.8 | 31.8 | 1.7 | 19.9 | 1.9 |
| 41 | 241.4 | 20.4 | 38.9 | 1.5 | 34.4 | 2.4 | 19.6 | 1.0 |
| 42 | 172.9 | 24.3 | 37.8 | 0.5 | 33.2 | 1.8 | 21.2 | 2.2 |
| 43 | 119.7 | 20.8 | 36.5 | 2.4 | 34.1 | 2.8 | 27.5 | 2.4 |
| 44 | 278.2 | 61.3 | 41.3 | 2.3 | 33.3 | 2.7 | 19.1 | 4.7 |
| 45 | 195.4 | 41.9 | 38.1 | 4.6 | 33.6 | 2.8 | 22.0 | 4.5 |

**Appendix N** Summary of values for selected seed constituent traits in desi chickpea (Continued).

| Genotype No. | TSW (g) | | Total starch (%) | | Amylose (%) | | Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 46 | 193.0 | 31.4 | 36.9 | 1.2 | 33.7 | 2.5 | 21.1 | 2.9 |
| 47 | 190.2 | 32.3 | 38.9 | 1.3 | 34.1 | 3.7 | 20.7 | 3.5 |
| 48 | 199.4 | 17.9 | 35.5 | 1.6 | 32.6 | 2.4 | 21.5 | 3.3 |
| 49 | 152.7 | 39.2 | 35.0 | 1.4 | 33.5 | 1.1 | 21.3 | 1.0 |
| 50 | 148.7 | 35.0 | 37.1 | 3.4 | 31.1 | 0.8 | 20.7 | 1.2 |
| 51 | 187.5 | 53.8 | 40.9 | 4.0 | 31.3 | 2.5 | 17.2 | 2.1 |
| 52 | 138.5 | 34.7 | 39.0 | 4.7 | 32.9 | 3.1 | 22.0 | 4.1 |
| 53 | 144.7 | 27.2 | 37.4 | 2.8 | 32.2 | 2.0 | 22.0 | 2.3 |
| 54 | 277.6 | 21.4 | 37.4 | 3.0 | 33.4 | 4.0 | 21.8 | 4.3 |
| 55 | 178.0 | 25.3 | 35.2 | 3.4 | 32.0 | 2.1 | 23.0 | 5.6 |
| 56 | 128.2 | 31.8 | 34.4 | 0.9 | 32.9 | 2.2 | 21.5 | 3.2 |
| 57 | 157.9 | 25.0 | 37.8 | 3.1 | 32.5 | 3.3 | 22.4 | 4.6 |
| 58 | 132.7 | 42.3 | 36.4 | 3.7 | 32.7 | 1.6 | 20.9 | 1.7 |
| 59 | 179.4 | 27.1 | 37.0 | 1.3 | 33.0 | 2.3 | 20.8 | 2.0 |
| 60 | 145.2 | 31.8 | 37.1 | 4.5 | 33.3 | 2.5 | 22.1 | 2.2 |
| 61 | 134.2 | 35.9 | 36.6 | 3.3 | 32.8 | 2.8 | 21.0 | 3.9 |
| 62 | 133.6 | 31.8 | 37.4 | 2.5 | 32.9 | 1.3 | 21.7 | 3.6 |
| 63 | 137.7 | 32.8 | 36.3 | 1.7 | 33.1 | 0.8 | 21.6 | 3.2 |
| 64 | 129.5 | 34.8 | 39.6 | 4.2 | 31.8 | 0.5 | 21.0 | 1.9 |
| 65 | 133.1 | 35.0 | 38.8 | 4.5 | 31.1 | 2.2 | 21.2 | 3.9 |
| 66 | 136.1 | 36.9 | 38.9 | 2.0 | 32.3 | 1.6 | 20.7 | 2.9 |
| 67 | 137.6 | 30.7 | 39.1 | 3.1 | 32.5 | 0.7 | 19.6 | 4.3 |
| 68 | 238.6 | 70.5 | 39.0 | 4.3 | 31.8 | 3.2 | 22.1 | 5.7 |
| 69 | 135.4 | 41.4 | 37.1 | 0.8 | 33.1 | 1.1 | 19.8 | 2.1 |
| 70 | 173.5 | 30.2 | 38.7 | 1.3 | 33.5 | 0.4 | 20.1 | 2.5 |
| 71 | 128.8 | 38.3 | 37.2 | 1.2 | 34.4 | 0.8 | 19.8 | 2.9 |
| 72 | 143.2 | 31.1 | 34.1 | 1.2 | 34.4 | 1.4 | 22.3 | 2.6 |
| 73 | 127.6 | 33.6 | 36.0 | 1.7 | 34.1 | 0.7 | 21.2 | 2.5 |
| 74 | 117.3 | 30.7 | 34.7 | 0.9 | 34.2 | 0.2 | 22.3 | 3.6 |
| 75 | 182.9 | 23.5 | 36.2 | 1.9 | 32.4 | 1.2 | 21.6 | 3.5 |
| 76 | 146.5 | 45.6 | 37.2 | 4.3 | 33.6 | 3.0 | 20.4 | 1.7 |
| 77 | 159.7 | 26.6 | 38.6 | 3.0 | 30.7 | 3.0 | 20.2 | 1.7 |
| 78 | 189.7 | 37.1 | 36.7 | 1.3 | 31.9 | 1.6 | 20.5 | 2.5 |
| 79 | 153.0 | 30.9 | 36.2 | 3.3 | 30.8 | 1.7 | 21.2 | 2.8 |
| 80 | 160.3 | 36.5 | 36.4 | 3.2 | 32.0 | 2.6 | 22.1 | 3.2 |
| 81 | 142.1 | 36.5 | 35.8 | 1.6 | 31.6 | 2.9 | 22.8 | 2.5 |
| 82 | 227.6 | 58.8 | 39.8 | 3.6 | 31.4 | 2.4 | 18.1 | 1.4 |
| 83 | 140.5 | 43.9 | 40.6 | 5.9 | 32.8 | 3.8 | 20.2 | 3.7 |
| 84 | 182.0 | 35.6 | 36.1 | 1.0 | 30.9 | 2.7 | 20.2 | 2.0 |
| 85 | 195.3 | 45.5 | 39.8 | 1.9 | 30.3 | 3.3 | 20.4 | 3.0 |
| 86 | 159.3 | 20.0 | 35.3 | 1.2 | 32.9 | 2.0 | 22.5 | 2.6 |
| 87 | 207.0 | 42.6 | 38.5 | 0.9 | 30.9 | 3.0 | 17.4 | 0.7 |
| 88 | 156.3 | 34.5 | 36.2 | 1.5 | 29.9 | 2.2 | 20.8 | 1.5 |
| 89 | 222.6 | 63.4 | 39.2 | 0.5 | 30.9 | 1.9 | 19.3 | 2.4 |
| 90 | 298.5 | 60.7 | 40.1 | 0.8 | 31.8 | 0.7 | 21.1 | 1.9 |

**Appendix N** Summary of values for selected seed constituent traits in desi chickpea (Continued).

| Genotype No. | TSW (g) | | Total starch (%) | | Amylose (%) | | Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 91 | 190.2 | 28.5 | 37.6 | 0.5 | 32.4 | 2.8 | 19.6 | 0.2 |
| 92 | 196.4 | 35.1 | 38.4 | 3.5 | 33.6 | 0.7 | 20.1 | 4.2 |
| 93 | 189.3 | 49.5 | 36.5 | 2.4 | 33.5 | 2.1 | 20.5 | 2.5 |
| 94 | 198.0 | 17.3 | 36.3 | 1.7 | 33.7 | 3.0 | 21.1 | 4.2 |
| 95 | 251.7 | 28.8 | 39.1 | 4.5 | 32.3 | 1.6 | 20.5 | 2.7 |
| 96 | 264.7 | 57.8 | 41.9 | 4.1 | 32.8 | 0.3 | 20.5 | 4.7 |
| 97 | 279.5 | 55.4 | 42.8 | 1.5 | 33.4 | 0.7 | 18.9 | 1.7 |
| 98 | 271.1 | 24.7 | 41.5 | 3.2 | 32.9 | 1.5 | 19.9 | 5.2 |
| 99 | 167.2 | 39.3 | 36.8 | 2.4 | 34.0 | 2.1 | 20.7 | 3.7 |
| 100 | 280.3 | 51.8 | 40.0 | 4.3 | 33.3 | 0.9 | 21.0 | 3.4 |
| 101 | 195.7 | 46.6 | 38.9 | 2.1 | 32.1 | 2.7 | 21.7 | 2.9 |
| 102 | 200.0 | 36.6 | 35.1 | 3.1 | 33.1 | 1.7 | 22.1 | 0.7 |
| 103 | 215.2 | 51.0 | 41.4 | 2.9 | 32.0 | 1.7 | 18.9 | 2.6 |
| 104 | 236.2 | 46.9 | 41.3 | 2.1 | 31.2 | 1.4 | 19.4 | 2.6 |
| 105 | 207.7 | 47.7 | 42.1 | 3.7 | 31.4 | 1.3 | 18.9 | 3.2 |
| 106 | 330.8 | 34.5 | 40.8 | 2.9 | 32.0 | 0.9 | 17.4 | 0.7 |
| 107 | 284.1 | 36.6 | 40.9 | 2.4 | 33.0 | 0.6 | 18.0 | 3.8 |
| 108 | 311.8 | 33.4 | 41.5 | 3.7 | 33.1 | 1.3 | 19.9 | 1.5 |
| 109 | 203.1 | 25.7 | 37.7 | 3.6 | 32.2 | 0.9 | 20.5 | 2.4 |
| 110 | 260.0 | 49.9 | 39.1 | 4.3 | 31.7 | 1.6 | 18.1 | 1.7 |
| 111 | 254.7 | 37.1 | 40.8 | 2.0 | 32.0 | 0.7 | 19.3 | 4.9 |
| 112 | 273.5 | 32.2 | 40.8 | 3.3 | 29.7 | 0.6 | 16.7 | 0.7 |
| 113 | 185.2 | 40.3 | 35.7 | 1.4 | 33.5 | 1.7 | 20.0 | 1.7 |
| 114 | 175.9 | 38.5 | 36.0 | 0.7 | 31.6 | 1.6 | 21.7 | 3.4 |
| 115 | 305.4 | 76.0 | 40.3 | 3.2 | 32.9 | 0.9 | 19.4 | 4.7 |
| 116 | 325.4 | 24.5 | 41.4 | 3.7 | 31.4 | 1.0 | 19.3 | 4.8 |
| 117 | 316.1 | 24.4 | 40.8 | 2.8 | 32.4 | 0.9 | 19.3 | 4.7 |
| 118 | 236.6 | 36.1 | 41.6 | 1.0 | 31.5 | 1.4 | 19.1 | 4.8 |
| 119 | 332.4 | 31.3 | 40.3 | 2.7 | 32.9 | 0.8 | 19.4 | 3.1 |
| 120 | 310.0 | 27.9 | 39.7 | 2.4 | 33.0 | 1.3 | 17.7 | 2.5 |
| 121 | 294.6 | 22.8 | 41.6 | 1.1 | 32.9 | 1.1 | 18.3 | 2.9 |
| 122 | 147.3 | 47.1 | 41.4 | 6.3 | 36.4 | 3.8 | 22.0 | 4.2 |
| 123 | 150.1 | 27.3 | 38.3 | 7.7 | 37.8 | 4.9 | 19.6 | 2.1 |
| 124 | 148.2 | 30.1 | 38.4 | 5.6 | 34.3 | 0.9 | 20.1 | 3.8 |
| 125 | 153.3 | 78.0 | 39.0 | 2.7 | 32.7 | 2.7 | 21.6 | 1.8 |
| 126 | 159.3 | 40.0 | 37.5 | 3.4 | 34.2 | 0.6 | 22.3 | 2.3 |
| 127 | 157.6 | 26.9 | 39.9 | 3.1 | 33.9 | 2.3 | 21.4 | 3.8 |
| 128 | 144.3 | 36.2 | 33.6 | 0.7 | 34.0 | 1.9 | 21.9 | 4.8 |
| 129 | 127.1 | 32.8 | 36.1 | 2.9 | 34.8 | 1.1 | 20.2 | 4.0 |
| 130 | 168.0 | 18.1 | 40.7 | 1.9 | 32.0 | 2.8 | 22.9 | 0.5 |
| 131 | 128.6 | 18.7 | 40.0 | 0.9 | 32.8 | 1.2 | 22.4 | 3.4 |
| 132 | 193.1 | 67.1 | 35.9 | 1.4 | 31.6 | 0.6 | 20.4 | 2.6 |
| 133 | 152.8 | 48.5 | 30.0 | 0.7 | 33.2 | 1.2 | 20.9 | 4.8 |
| 134 | 138.3 | 17.2 | 34.7 | 5.0 | 32.7 | 0.7 | 22.7 | 5.1 |
| 135 | 149.6 | 33.6 | 35.3 | 1.2 | 33.6 | 1.9 | 22.8 | 5.6 |

**Appendix N** Summary of values for selected seed constituent traits in desi chickpea (Continued).

| Genotype No. | TSW (g) | | Total starch (%) | | Amylose (%) | | Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 136 | 197.6 | 30.0 | 34.9 | 5.9 | 36.6 | 2.0 | 22.9 | 5.3 |
| 137 | 181.4 | 52.9 | 37.4 | 6.6 | 35.7 | 0.2 | 20.9 | 4.0 |
| 138 | 198.8 | 53.3 | 40.9 | 1.9 | 33.6 | 0.1 | 19.7 | 6.0 |
| 139 | 159.5 | 48.5 | 40.6 | 2.3 | 33.4 | 1.7 | 21.9 | 2.2 |
| 140 | 188.3 | 53.2 | 41.1 | 1.8 | 33.9 | 2.0 | 20.5 | 3.4 |
| 141 | 230.7 | 60.5 | 38.5 | 1.6 | 37.0 | 3.2 | 22.1 | 5.2 |
| 142 | 194.1 | 62.2 | 36.7 | 2.1 | 35.1 | 0.8 | 22.6 | 4.7 |
| 143 | 192.3 | 18.1 | 36.3 | 1.9 | 37.1 | 0.4 | 20.7 | 3.0 |
| 144 | 141.7 | 31.4 | 35.4 | 4.5 | 33.8 | 3.7 | 21.9 | 2.9 |
| 145 | 129.0 | 15.4 | 37.8 | 1.9 | 33.8 | 1.5 | 21.4 | 2.9 |
| 146 | 127.3 | 55.3 | 36.4 | 0.4 | 34.3 | 2.5 | 19.3 | 5.9 |
| 147 | 167.3 | 22.3 | 38.1 | 2.4 | 34.3 | 2.9 | 22.8 | 4.1 |
| 148 | 243.9 | 20.5 | 38.6 | 4.0 | 32.5 | 2.0 | 21.1 | 6.1 |
| 149 | 151.3 | 17.6 | 40.8 | 2.3 | 34.1 | 1.8 | 20.9 | 5.6 |
| 150 | 150.4 | 43.0 | 39.1 | 2.3 | 32.9 | 2.4 | 21.8 | 2.6 |
| 151 | 133.2 | 32.8 | 37.9 | 5.0 | 34.4 | 2.2 | 22.6 | 3.3 |
| 152 | 135.8 | 20.2 | 40.0 | 1.3 | 34.6 | 0.8 | 21.9 | 3.2 |
| 153 | 132.3 | 5.7 | 34.4 | 3.7 | 35.2 | 1.2 | 23.3 | 0.9 |
| 154 | 141.5 | 24.7 | 39.3 | 4.8 | 34.5 | 2.2 | 21.8 | 4.3 |
| 155 | 174.2 | 25.0 | 40.0 | 3.2 | 33.6 | 3.2 | 21.4 | 2.6 |
| 156 | 123.6 | 29.0 | 40.8 | 2.6 | 33.6 | 3.0 | 21.1 | 3.6 |
| 157 | 164.7 | 12.2 | 38.3 | 2.6 | 34.2 | 3.8 | 23.4 | 4.4 |
| 158 | 131.3 | 31.9 | 39.7 | 2.7 | 33.6 | 0.8 | 20.8 | 4.0 |
| 159 | 115.9 | 10.4 | 38.3 | 2.0 | 33.7 | 4.5 | 21.6 | 4.5 |
| 160 | 128.1 | 10.4 | 38.6 | 3.4 | 34.3 | 2.1 | 21.6 | 4.9 |
| 161 | 151.1 | 21.9 | 37.0 | 1.3 | 34.7 | 1.8 | 20.8 | 3.5 |
| 162 | 207.6 | 14.4 | 41.9 | 1.8 | 34.1 | 1.7 | 21.2 | 4.6 |
| 163 | 124.3 | 4.5 | 37.9 | 1.1 | 32.0 | 3.8 | 21.6 | 5.1 |
| 164 | 124.0 | 4.5 | 39.7 | 3.3 | 34.7 | 3.0 | 20.7 | 3.2 |
| 165 | 199.0 | 23.7 | 42.2 | 1.9 | 34.9 | 1.6 | 19.8 | 5.3 |
| 166 | 153.0 | 38.4 | 37.7 | 2.3 | 33.7 | 3.6 | 21.5 | 3.3 |
| 167 | 118.4 | 22.5 | 35.4 | 2.8 | 35.4 | 4.8 | 22.0 | 5.5 |
| 168 | 143.4 | 24.4 | 37.2 | 2.9 | 35.4 | 3.3 | 21.8 | 5.1 |
| 169 | 131.1 | 21.6 | 39.9 | 2.7 | 34.0 | 4.4 | 21.0 | 3.3 |
| 170 | 131.7 | 17.8 | 40.0 | 3.3 | 35.5 | 3.9 | 20.6 | 4.6 |
| 171 | 112.2 | 12.9 | 39.1 | 0.8 | 33.9 | 3.4 | 21.7 | 5.8 |
| 172 | 135.2 | 18.9 | 40.5 | 2.1 | 35.4 | 4.5 | 21.4 | 4.1 |
| 173 | 114.4 | 20.3 | 39.2 | 2.1 | 33.2 | 4.1 | 21.0 | 3.0 |
| 174 | 142.0 | 30.0 | 41.4 | 1.0 | 36.0 | 4.2 | 22.0 | 1.6 |
| 175 | 108.6 | 14.4 | 37.9 | 4.8 | 35.0 | 2.9 | 21.0 | 3.8 |
| 176 | 131.3 | 3.5 | 39.0 | 2.6 | 32.8 | 2.3 | 21.2 | 5.1 |
| 177 | 122.5 | 18.8 | 41.6 | 2.4 | 34.7 | 3.1 | 21.0 | 2.6 |
| 178 | 131.2 | 21.6 | 40.3 | 2.0 | 34.9 | 2.0 | 20.1 | 5.6 |
| 179 | 134.5 | 16.4 | 41.7 | 1.2 | 33.0 | 1.2 | 20.3 | 4.8 |
| 180 | 142.3 | 25.3 | 39.6 | 2.0 | 33.7 | 2.7 | 20.8 | 3.2 |

**Appendix N** Summary of values for selected seed constituent traits in desi chickpea (Continued).

| Genotype No. | TSW (g) | | Total starch (%) | | Amylose (%) | | Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 181 | 175.1 | 17.6 | 39.9 | 8.9 | 33.8 | 2.3 | 19.5 | 4.0 |
| 182 | 195.2 | 11.7 | 37.9 | 4.1 | 32.7 | 1.2 | 22.2 | 0.7 |
| 183 | 172.6 | 27.0 | 39.9 | 4.1 | 35.6 | 3.6 | 21.2 | 4.0 |
| 184 | 144.9 | 42.3 | 35.9 | 5.0 | 34.1 | 2.7 | 21.2 | 2.0 |
| 185 | 136.6 | 37.0 | 33.1 | 7.9 | 33.1 | 2.0 | 21.3 | 2.3 |
| 186 | 136.4 | 17.3 | 36.5 | 7.3 | 32.8 | 1.5 | 22.6 | 0.7 |
| 187 | 216.3 | 26.6 | 40.3 | 2.0 | 32.6 | 1.0 | 19.9 | 4.2 |
| 188 | 119.9 | 21.7 | 35.4 | 4.0 | 35.4 | 2.0 | 21.7 | 2.5 |
| 189 | 125.2 | 19.3 | 36.6 | 3.7 | 34.4 | 1.8 | 22.9 | 1.6 |
| 190 | 171.1 | 36.3 | 27.1 | 2.5 | 37.1 | 4.0 | 20.5 | 4.2 |
| 191 | 134.2 | 18.8 | 39.4 | 4.9 | 37.1 | 2.4 | 21.1 | 4.1 |
| 192 | 263.3 | 3.5 | 33.9 | 1.5 | 35.8 | 2.5 | 22.9 | 1.5 |
| 193 | 124.8 | 27.4 | 38.5 | 0.9 | 36.8 | 3.6 | 21.7 | 1.7 |
| 194 | 138.2 | 26.3 | 37.6 | 4.7 | 35.9 | 2.2 | 20.3 | 3.0 |
| 195 | 128.9 | 23.5 | 38.7 | 2.4 | 36.3 | 3.4 | 20.5 | 0.5 |
| 196 | 159.2 | 21.1 | 33.9 | 4.8 | 36.9 | 1.5 | 22.6 | 2.6 |
| 197 | 171.5 | 39.3 | 39.0 | 4.9 | 36.5 | 3.3 | 20.9 | 3.1 |
| 198 | 137.0 | 26.2 | 37.2 | 2.3 | 35.3 | 4.1 | 20.6 | 3.9 |
| 199 | 138.4 | 27.3 | 35.9 | 2.8 | 37.6 | 3.2 | 19.6 | 3.3 |
| 200 | 196.0 | 53.4 | 39.9 | 1.0 | 35.7 | 3.2 | 19.8 | 3.1 |
| 201 | 247.6 | 3.7 | 36.3 | 6.1 | 34.7 | 3.5 | 20.9 | 3.3 |
| 202 | 117.3 | 5.0 | 38.2 | 4.7 | 36.6 | 5.3 | 20.7 | 1.1 |
| 203 | 195.8 | 33.6 | 39.4 | 2.9 | 35.9 | 3.5 | 21.0 | 5.0 |
| 204 | 102.8 | 9.6 | 40.7 | 1.5 | 35.6 | 3.7 | 19.7 | 5.3 |
| 205 | 166.4 | 9.0 | 36.3 | 5.7 | 34.4 | 3.1 | 19.2 | 4.6 |
| 206 | 179.7 | 8.3 | 37.8 | 4.3 | 34.0 | 2.8 | 19.8 | 3.8 |
| 207 | 148.6 | 14.3 | 37.6 | 5.0 | 34.4 | 3.3 | 19.6 | 2.1 |
| 208 | 241.4 | 23.1 | 40.8 | 2.0 | 33.8 | 4.2 | 18.8 | 3.3 |
| 209 | 226.4 | 20.3 | 42.2 | 3.9 | 34.3 | 3.5 | 20.1 | 5.4 |
| 210 | 197.4 | 14.8 | 42.8 | 4.2 | 35.9 | 3.3 | 19.4 | 3.4 |
| 211 | 154.2 | 21.3 | 39.4 | 3.4 | 36.2 | 4.7 | 20.6 | 4.7 |
| 212 | 239.1 | 10.2 | 38.5 | 3.6 | 35.9 | 3.8 | 19.9 | 2.9 |
| 213 | 194.9 | 10.2 | 37.6 | 4.0 | 38.0 | 5.2 | 19.3 | 4.2 |
| 214 | 145.5 | 16.7 | 38.3 | 2.9 | 35.5 | 4.2 | 20.3 | 2.4 |
| 215 | 167.6 | 25.8 | 38.4 | 2.4 | 34.6 | 3.4 | 20.0 | 4.7 |
| 216 | 239.0 | 14.2 | 43.0 | 2.8 | 36.0 | 2.5 | 19.8 | 4.9 |
| 217 | 185.4 | 13.0 | 39.0 | 3.0 | 35.8 | 2.7 | 21.3 | 4.4 |
| 218 | 126.7 | 12.3 | 37.5 | 1.8 | 34.9 | 2.7 | 22.5 | 5.4 |
| 219 | 187.0 | 53.2 | 34.3 | 1.8 | 35.6 | 3.4 | 18.3 | 3.8 |
| 220 | 126.3 | 10.2 | 35.3 | 3.2 | 36.7 | 3.4 | 21.2 | 6.4 |
| 221 | 121.4 | 21.2 | 34.5 | 5.3 | 35.4 | 5.3 | 22.6 | 0.9 |
| 222 | 121.7 | 9.8 | 36.7 | 4.4 | 36.9 | 4.3 | 20.7 | 1.8 |
| 223 | 142.8 | 21.4 | 40.6 | 0.9 | 35.7 | 4.0 | 20.3 | 4.7 |
| 224 | 165.6 | 40.0 | 38.1 | 2.4 | 37.0 | 3.5 | 20.9 | 4.7 |
| 225 | 171.0 | 18.5 | 38.9 | 1.5 | 38.6 | 2.3 | 20.2 | 2.7 |

**Appendix N** Summary of values for selected seed constituent traits in desi chickpea (Continued).

| Genotype No. | TSW (g) | | Total starch (%) | | Amylose (%) | | Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 226 | 150.9 | 12.3 | 38.8 | 2.5 | 34.1 | 2.1 | 19.7 | 4.0 |
| 227 | 172.4 | 21.9 | 39.3 | 3.1 | 36.8 | 3.0 | 19.3 | 3.7 |
| 228 | 180.1 | 69.1 | 37.3 | 3.3 | 35.9 | 3.3 | 20.9 | 3.3 |
| 229 | 183.7 | 12.0 | 38.0 | 3.4 | 34.4 | 2.9 | 21.8 | 4.2 |
| 230 | 140.1 | 30.2 | 38.2 | 2.3 | 35.9 | 4.2 | 20.3 | 3.1 |
| 231 | 136.0 | 24.1 | 39.3 | 3.1 | 36.2 | 4.6 | 20.0 | 3.6 |
| 232 | 170.2 | 25.3 | 40.4 | 1.6 | 35.1 | 3.0 | 19.8 | 3.7 |
| 233 | 164.8 | 15.0 | 35.4 | 6.0 | 36.0 | 4.9 | 18.5 | 4.7 |
| 234 | 99.8 | 10.3 | 37.1 | 5.9 | 36.5 | 5.2 | 20.5 | 4.8 |
| 235 | 103.3 | 4.3 | 35.4 | 7.3 | 35.4 | 4.0 | 20.4 | 6.0 |
| 236 | 130.2 | 16.6 | 34.6 | 2.8 | 37.2 | 1.1 | 20.6 | 0.8 |
| 237 | 143.6 | 35.7 | 34.9 | 2.1 | 36.5 | 3.2 | 20.1 | 2.6 |
| 238 | 147.9 | 52.0 | 42.0 | 7.3 | 34.4 | 2.4 | 20.5 | 1.6 |
| 239 | 139.7 | 43.2 | 39.5 | 2.9 | 36.5 | 3.3 | 20.9 | 2.6 |
| 240 | 136.9 | 47.9 | 39.5 | 6.5 | 36.1 | 2.7 | 20.6 | 1.9 |
| 241 | 164.0 | 14.4 | 42.6 | 1.7 | 35.7 | 3.0 | 21.1 | 2.7 |
| 242 | 159.5 | 49.8 | 39.5 | 3.4 | 35.0 | 2.1 | 19.8 | 1.1 |
| 243 | 146.7 | 49.5 | 38.0 | 2.3 | 35.8 | 5.1 | 19.9 | 1.6 |
| 244 | 149.1 | 32.8 | 39.8 | 2.6 | 35.2 | 3.4 | 21.1 | 4.6 |
| 245 | 160.3 | 42.6 | 41.1 | 4.2 | 34.9 | 3.6 | 21.0 | 2.3 |
| 246 | 161.3 | 41.0 | 41.7 | 3.8 | 35.0 | 1.4 | 20.5 | 2.1 |
| 247 | 148.9 | 24.4 | 40.2 | 4.5 | 34.3 | 1.8 | 21.7 | 0.9 |
| 248 | 222.5 | 31.2 | 43.3 | 0.5 | 32.9 | 2.5 | 19.3 | 4.4 |
| 249 | 154.0 | 32.2 | 38.6 | 3.3 | 35.4 | 2.2 | 21.1 | 1.6 |
| 250 | 133.3 | 43.7 | 42.2 | 13.6 | 34.0 | 6.1 | 21.5 | 0.8 |
| 251 | 195.9 | 19.5 | 44.1 | 5.2 | 33.4 | 4.2 | 22.0 | 2.1 |
| 252 | 310.6 | 22.6 | 45.8 | 5.3 | 33.3 | 4.1 | 18.1 | 3.5 |
| 253 | 132.5 | 14.7 | 40.2 | 1.4 | 31.7 | 4.9 | 20.0 | 3.1 |
| 254 | 137.7 | 22.2 | 44.1 | 7.5 | 33.1 | 4.3 | 19.3 | 2.7 |
| 255 | 136.9 | 33.1 | 43.0 | 6.2 | 33.7 | 4.0 | 21.0 | 2.4 |
| 256 | 143.5 | 35.3 | 42.1 | 2.4 | 32.7 | 4.8 | 19.8 | 3.5 |
| 257 | 130.1 | 11.5 | 40.5 | 4.6 | 32.4 | 4.6 | 19.6 | 2.9 |
| 258 | 146.4 | 32.6 | 42.4 | 3.4 | 34.1 | 3.3 | 21.4 | 3.2 |
| 259 | 149.6 | 22.1 | 42.1 | 0.6 | 34.2 | 4.5 | 21.2 | 2.3 |
| 260 | 153.4 | 32.2 | 40.6 | 3.1 | 34.9 | 3.4 | 20.1 | 2.2 |
| 261 | 191.5 | 63.8 | 44.2 | 5.2 | 33.9 | 3.1 | 21.1 | 1.1 |
| 262 | 146.6 | 26.8 | 38.5 | 2.3 | 34.9 | 3.8 | 19.7 | 2.2 |
| 263 | 130.3 | 11.5 | 44.9 | 9.9 | 34.5 | 2.6 | 20.5 | 3.0 |
| 264 | 220.3 | 24.2 | 40.3 | 9.5 | 33.7 | 3.3 | 21.9 | 3.1 |
| 265 | 146.3 | 28.9 | 40.6 | 2.7 | 33.2 | 3.2 | 21.6 | 2.8 |
| 266 | 128.6 | 21.4 | 42.2 | 2.0 | 33.5 | 2.6 | 19.9 | 2.7 |
| 267 | 145.6 | 39.2 | 40.8 | 4.9 | 33.2 | 3.3 | 20.6 | 0.4 |
| 268 | 112.8 | 13.2 | 38.4 | 1.6 | 33.1 | 4.1 | 18.6 | 5.1 |
| 269 | 170.0 | 1.9 | 40.2 | 3.3 | 35.6 | 4.8 | 20.0 | 3.7 |
| 270 | 164.0 | 20.4 | 34.8 | 9.4 | 33.4 | 4.7 | 21.2 | 1.6 |

**Appendix N** Summary of values for selected seed constituent traits in desi chickpea (Continued).

| Genotype No. | TSW (g) | | Total starch (%) | | Amylose (%) | | Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 271 | 169.8 | 18.9 | 33.5 | 9.0 | 32.9 | 4.0 | 22.2 | 3.2 |
| 272 | 178.4 | 17.6 | 35.9 | 3.6 | 33.8 | 3.6 | 20.4 | 4.2 |
| 273 | 126.7 | 10.6 | 38.6 | 1.6 | 35.0 | 2.5 | 20.2 | 3.1 |
| 274 | 153.1 | 16.2 | 37.1 | 2.5 | 35.2 | 2.1 | 21.5 | 3.1 |
| 275 | 151.0 | 16.5 | 38.4 | 0.6 | 34.4 | 1.4 | 20.8 | 3.2 |
| 276 | 161.6 | 24.2 | 38.3 | 3.6 | 34.4 | 1.3 | 19.9 | 2.6 |
| 277 | 205.9 | 11.2 | 42.6 | 4.1 | 35.5 | 0.5 | 19.0 | 4.1 |
| 278 | 243.5 | 6.7 | 42.9 | 3.0 | 35.8 | 2.5 | 19.9 | 3.9 |
| 279 | 223.0 | 20.8 | 42.1 | 4.3 | 35.3 | 2.1 | 20.6 | 3.4 |
| 280 | 171.6 | 14.2 | 40.3 | 7.6 | 35.2 | 2.6 | 20.2 | 5.7 |
| 281 | 149.7 | 15.9 | 39.1 | 5.2 | 36.1 | 3.0 | 21.6 | 4.1 |
| 282 | 226.5 | 28.0 | 44.9 | 3.0 | 34.9 | 3.1 | 19.7 | 4.2 |
| 283 | 177.9 | 14.2 | 37.1 | 3.0 | 35.5 | 2.9 | 20.2 | 3.9 |
| 284 | 138.5 | 11.0 | 38.7 | 5.0 | 34.2 | 4.4 | 20.9 | 4.8 |
| 285 | 183.6 | 24.3 | 39.3 | 4.8 | 36.0 | 2.9 | 20.0 | 3.5 |
| 286 | 295.0 | 30.3 | 45.2 | 4.0 | 35.0 | 2.4 | 20.3 | 3.6 |
| 287 | 158.8 | 5.4 | 42.0 | 6.0 | 36.1 | 3.7 | 21.3 | 5.2 |
| 288 | 141.0 | 35.7 | 39.5 | 8.5 | 35.2 | 2.2 | 20.8 | 4.0 |
| 289 | 160.5 | 31.3 | 40.6 | 11.9 | 35.3 | 3.8 | 19.9 | 2.0 |
| 290 | 137.7 | 15.9 | 39.1 | 4.0 | 34.6 | 3.5 | 21.1 | 3.9 |
| 291 | 150.5 | 26.6 | 39.4 | 7.2 | 35.8 | 2.7 | 22.2 | 5.5 |
| 292 | 175.6 | 15.9 | 41.3 | 7.7 | 35.8 | 0.7 | 21.4 | 4.1 |
| 293 | 144.5 | 15.3 | 38.7 | 6.8 | 35.4 | 2.9 | 21.2 | 3.6 |
| 294 | 181.8 | 15.6 | 44.4 | 1.9 | 36.6 | 1.6 | 21.5 | 4.4 |
| 295 | 184.0 | 27.7 | 43.2 | 7.5 | 36.1 | 1.8 | 22.2 | 3.2 |
| 296 | 222.7 | 54.7 | 43.7 | 2.1 | 33.5 | 2.9 | 18.6 | 2.2 |
| 297 | 192.0 | 50.6 | 34.5 | 4.8 | 33.0 | 3.0 | 19.0 | 3.0 |
| 298 | 180.1 | 59.1 | 35.2 | 0.3 | 33.2 | 3.6 | 18.9 | 3.6 |
| 299 | 163.7 | 23.6 | 38.0 | 0.5 | 36.4 | 2.4 | 17.2 | 0.7 |
| 300 | 133.5 | 10.5 | 38.7 | 1.0 | 32.6 | 1.2 | 23.2 | 2.1 |
| 301 | 123.9 | 28.3 | 41.1 | 3.3 | 32.0 | 2.5 | 20.1 | 2.7 |
| 302 | 192.2 | 48.8 | 36.9 | 3.3 | 32.6 | 3.2 | 19.3 | 3.1 |
| 303 | 184.2 | 57.4 | 35.1 | 5.1 | 34.4 | 2.6 | 20.4 | 1.3 |
| 304 | 119.2 | 40.7 | 33.1 | 6.5 | 32.3 | 2.7 | 21.9 | 2.9 |
| 305 | 230.5 | 99.2 | 44.5 | 6.5 | 32.0 | 2.2 | 16.9 | 3.7 |
| 306 | 204.1 | 31.7 | 42.1 | 2.0 | 32.1 | 3.1 | 16.8 | 3.0 |
| 307 | 219.5 | 62.2 | 41.3 | 2.9 | 33.5 | 3.1 | 17.5 | 2.2 |
| 308 | 130.2 | 50.1 | 38.8 | 1.3 | 33.4 | 3.0 | 20.5 | 2.5 |
| 309 | 111.3 | 32.5 | 36.0 | 2.7 | 33.4 | 2.7 | 22.3 | 2.2 |
| 310 | 135.5 | 33.9 | 33.7 | 11.1 | 32.0 | 3.5 | 20.4 | 2.9 |
| 311 | 148.4 | 17.1 | 42.7 | 0.9 | 34.6 | 2.6 | 21.9 | 0.8 |
| 312 | 169.5 | 50.5 | 39.3 | 1.5 | 32.8 | 3.9 | 20.2 | 2.3 |
| 313 | 181.5 | 46.2 | 41.2 | 2.9 | 32.9 | 4.9 | 18.7 | 2.8 |
| 314 | 194.0 | 65.4 | 38.1 | 1.0 | 33.4 | 4.4 | 18.0 | 3.1 |
| 315 | 174.9 | 68.9 | 38.2 | 4.7 | 32.7 | 4.1 | 19.1 | 2.5 |

**Appendix N** Summary of values for selected seed constituent traits in desi chickpea (Continued).

| Genotype No. | TSW (g) | | Total starch (%) | | Amylose (%) | | Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 316 | 228.7 | 77.1 | 42.4 | 2.0 | 31.7 | 3.8 | 17.9 | 2.7 |
| 317 | 167.7 | 43.9 | 38.0 | 2.8 | 32.2 | 4.4 | 17.2 | 3.4 |
| 318 | 208.5 | 59.6 | 43.0 | 0.1 | 32.5 | 3.6 | 18.5 | 2.5 |
| 319 | 218.5 | 76.5 | 41.8 | 3.4 | 31.9 | 4.1 | 17.0 | 2.3 |
| 320 | 189.5 | 51.2 | 33.8 | 5.9 | 32.7 | 3.6 | 17.8 | 2.3 |
| 321 | 192.5 | 42.4 | 39.4 | 5.0 | 31.7 | 3.3 | 16.7 | 2.7 |
| 322 | 164.3 | 36.6 | 43.2 | 1.3 | 32.6 | 4.2 | 20.0 | 2.6 |
| 323 | 195.0 | 61.7 | 36.8 | 3.7 | 32.4 | 3.4 | 19.3 | 2.5 |
| 324 | 174.1 | 34.4 | 40.5 | 2.6 | 31.4 | 3.7 | 17.0 | 2.2 |
| 325 | 148.8 | 45.4 | 38.1 | 6.7 | 32.1 | 3.9 | 19.3 | 2.5 |
| 326 | 200.3 | 69.4 | 43.2 | 3.2 | 31.8 | 4.3 | 19.0 | 2.5 |
| 327 | 185.2 | 57.4 | 45.2 | 2.3 | 31.7 | 3.6 | 17.0 | 1.6 |
| 328 | 197.9 | 58.2 | 40.0 | 3.2 | 31.2 | 4.0 | 17.5 | 1.9 |
| 329 | 184.0 | 46.1 | 44.6 | 1.4 | 33.1 | 4.5 | 17.3 | 2.7 |
| 330 | 170.4 | 52.0 | 41.0 | 3.1 | 32.0 | 3.7 | 18.1 | 2.5 |
| 331 | 210.4 | 87.3 | 43.5 | 1.4 | 31.6 | 3.6 | 16.3 | 2.1 |
| 332 | 222.0 | 96.9 | 41.0 | 2.4 | 31.2 | 3.8 | 16.8 | 2.9 |
| 333 | 155.8 | 39.0 | 38.0 | 2.8 | 31.6 | 4.5 | 17.3 | 3.0 |
| 334 | 100.3 | 27.8 | 40.3 | 3.8 | 32.0 | 3.6 | 19.6 | 2.2 |
| 335 | 178.9 | 64.7 | 38.3 | 1.3 | 31.2 | 3.6 | 18.4 | 2.0 |
| 336 | 92.5 | 35.9 | 36.7 | 6.4 | 31.9 | 3.3 | 22.8 | 3.4 |
| 337 | 161.1 | 58.9 | 38.0 | 7.6 | 32.1 | 2.9 | 21.0 | 0.3 |
| 338 | 112.2 | 47.2 | 41.0 | 3.6 | 31.5 | 3.2 | 21.5 | 1.9 |
| 339 | 211.7 | 59.2 | 41.5 | 1.6 | 30.7 | 3.3 | 18.6 | 2.4 |
| 340 | 194.8 | 45.5 | 42.6 | 3.2 | 32.1 | 3.2 | 16.5 | 3.0 |
| 341 | 181.7 | 71.0 | 38.5 | 5.1 | 32.7 | 4.2 | 18.3 | 2.2 |
| 342 | 200.9 | 67.2 | 42.4 | 2.2 | 30.4 | 3.8 | 17.7 | 2.2 |
| 343 | 191.0 | 59.6 | 40.4 | 5.0 | 32.8 | 3.2 | 18.0 | 3.0 |
| 344 | 191.2 | 53.0 | 43.2 | 3.3 | 30.8 | 3.4 | 17.2 | 2.3 |
| 345 | 183.8 | 78.6 | 38.7 | 2.7 | 31.6 | 3.1 | 19.4 | 2.0 |
| 346 | 214.6 | 77.0 | 40.4 | 1.9 | 31.3 | 2.8 | 17.2 | 2.0 |
| 347 | 240.5 | 57.4 | 43.2 | 2.5 | 31.3 | 2.9 | 16.5 | 2.5 |
| 348 | 194.6 | 23.1 | 41.9 | 1.7 | 31.0 | 4.5 | 18.9 | 1.2 |
| 349 | 159.1 | 54.2 | 41.6 | 3.5 | 32.2 | 3.5 | 17.5 | 1.5 |
| 350 | 143.3 | 37.3 | 38.5 | 1.4 | 32.4 | 3.6 | 18.2 | 1.2 |
| 351 | 95.8 | 39.7 | 41.0 | 2.3 | 31.4 | 3.4 | 21.1 | 2.3 |
| 352 | 185.9 | 55.6 | 39.6 | 4.6 | 31.9 | 3.9 | 17.2 | 2.3 |
| 353 | 117.3 | 56.1 | 35.3 | 4.9 | 32.0 | 4.6 | 23.1 | 1.8 |
| 354 | 208.4 | 58.8 | 44.4 | 4.8 | 31.7 | 3.5 | 17.8 | 2.1 |
| 355 | 171.1 | 49.0 | 44.0 | 4.5 | 32.9 | 3.4 | 18.0 | 2.2 |
| 356 | 177.7 | 58.2 | 38.8 | 2.7 | 33.5 | 3.5 | 19.8 | 2.7 |
| 357 | 201.3 | 27.1 | 39.5 | 1.5 | 34.8 | 3.6 | 17.9 | 2.4 |
| 358 | 164.1 | 29.4 | 39.1 | 3.0 | 33.9 | 2.9 | 17.3 | 2.5 |
| 359 | 115.4 | 32.1 | 39.3 | 2.9 | 34.1 | 3.2 | 22.6 | 1.9 |
| 360 | 102.1 | 39.5 | 37.2 | 5.8 | 34.0 | 2.1 | 22.1 | 1.8 |

**Appendix N** Summary of values for selected seed constituent traits in desi chickpea (Continued).

| Genotype No. | TSW (g) | | Total starch (%) | | Amylose (%) | | Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 361 | 82.6 | 24.6 | 34.3 | 3.1 | 33.4 | 3.5 | 21.6 | 2.1 |
| 362 | 97.5 | 44.0 | 34.3 | 3.0 | 33.3 | 4.7 | 24.4 | 1.9 |
| 363 | 191.9 | 37.1 | 38.1 | 2.3 | 33.9 | 2.9 | 18.5 | 1.6 |
| 364 | 178.3 | 66.5 | 38.6 | 2.2 | 32.5 | 3.7 | 16.2 | 1.6 |
| 365 | 123.0 | 47.3 | 35.2 | 5.8 | 34.3 | 4.0 | 22.5 | 2.0 |
| 366 | 158.8 | 26.4 | 37.5 | 3.8 | 32.5 | 4.1 | 17.7 | 1.3 |
| 367 | 207.5 | 53.4 | 40.0 | 2.4 | 33.9 | 3.4 | 17.8 | 3.0 |
| 368 | 218.9 | 87.6 | 41.2 | 1.1 | 31.9 | 4.0 | 16.7 | 2.0 |
| 369 | 111.2 | 21.5 | 41.2 | 2.1 | 31.8 | 4.9 | 19.4 | 2.7 |
| 370 | 184.7 | 43.2 | 37.9 | 1.6 | 33.9 | 3.8 | 17.9 | 2.5 |
| 371 | 213.3 | 60.1 | 43.5 | 2.0 | 31.9 | 4.4 | 17.9 | 0.8 |
| 372 | 157.2 | 39.0 | 37.8 | 1.5 | 34.2 | 4.6 | 17.2 | 2.4 |
| 373 | 129.4 | 49.7 | 42.9 | 3.3 | 32.7 | 5.5 | 20.2 | 2.1 |
| 374 | 102.2 | 48.8 | 33.6 | 4.2 | 33.6 | 3.6 | 22.3 | 1.5 |
| 375 | 155.9 | 43.9 | 41.4 | 3.0 | 31.6 | 4.3 | 20.1 | 2.5 |
| 376 | 191.7 | 56.0 | 38.1 | 3.1 | 31.3 | 4.4 | 17.7 | 2.7 |
| 377 | 138.0 | 40.6 | 43.6 | 2.4 | 30.7 | 5.2 | 18.3 | 2.7 |
| 378 | 119.7 | 46.8 | 38.7 | 1.6 | 33.6 | 5.0 | 21.3 | 2.6 |
| 379 | 176.7 | 67.1 | 35.9 | 7.5 | 32.9 | 4.2 | 17.7 | 2.9 |
| 380 | 203.1 | 65.0 | 45.7 | 0.4 | 29.9 | 4.2 | 16.4 | 2.3 |
| 381 | 180.3 | 64.2 | 42.0 | 2.8 | 31.7 | 4.3 | 16.9 | 2.5 |
| 382 | 161.4 | 67.1 | 36.3 | 7.3 | 33.0 | 4.5 | 19.8 | 2.0 |
| 383 | 176.0 | 68.7 | 38.9 | 3.1 | 32.6 | 4.6 | 19.2 | 2.3 |
| 384 | 127.4 | 41.4 | 41.5 | 2.3 | 31.1 | 3.7 | 20.5 | 1.3 |
| 385 | 152.3 | 36.3 | 38.4 | 1.9 | 32.4 | 3.4 | 17.9 | 2.1 |
| 386 | 171.8 | 16.7 | 45.0 | 1.6 | 32.8 | 3.2 | 18.4 | 2.7 |
| 387 | 188.2 | 45.0 | 38.9 | 1.6 | 33.8 | 3.9 | 17.4 | 1.1 |
| 388 | 208.2 | 56.6 | 42.5 | 3.4 | 32.3 | 3.2 | 16.8 | 2.1 |
| 389 | 118.4 | 34.8 | 42.8 | 2.8 | 32.7 | 2.9 | 18.6 | 2.7 |
| 390 | 146.2 | 39.2 | 34.8 | 2.6 | 32.1 | 3.2 | 18.2 | 1.6 |
| 391 | 98.0 | 41.5 | 36.1 | 4.7 | 31.8 | 3.4 | 22.3 | 1.4 |
| 392 | 168.4 | 55.3 | 41.5 | 2.5 | 33.3 | 2.8 | 18.8 | 1.9 |
| 393 | 173.3 | 56.6 | 38.1 | 1.1 | 33.9 | 2.7 | 16.9 | 1.5 |
| 394 | 176.0 | 44.6 | 37.4 | 1.9 | 32.6 | 3.8 | 17.5 | 2.1 |
| 395 | 181.6 | 74.7 | 43.5 | 4.1 | 31.4 | 3.1 | 17.3 | 1.0 |
| 396 | 182.2 | 42.8 | 45.8 | 2.3 | 32.4 | 2.4 | 17.0 | 2.1 |
| 397 | 213.6 | 36.9 | 44.3 | 1.8 | 31.2 | 3.2 | 16.9 | 3.3 |
| 398 | 277.8 | 35.3 | 45.3 | 2.8 | 30.7 | 4.0 | 18.4 | 2.1 |
| 399 | 193.6 | 57.7 | 36.6 | 2.5 | 30.8 | 1.1 | 19.5 | 1.0 |
| 400 | 195.9 | 41.3 | 39.9 | 2.7 | 32.2 | 2.7 | 17.9 | 2.9 |
| 401 | 156.9 | 37.8 | 42.5 | 1.1 | 31.8 | 3.9 | 19.8 | 3.6 |
| 402 | 189.4 | 46.6 | 43.5 | 1.7 | 31.5 | 4.3 | 18.8 | 2.0 |
| 403 | 238.2 | 80.0 | 45.7 | 2.3 | 31.2 | 3.4 | 17.1 | 1.9 |
| 404 | 108.4 | 35.4 | 38.0 | 4.8 | 32.0 | 3.8 | 21.2 | 2.4 |
| 405 | 169.9 | 63.9 | 35.5 | 10.2 | 32.5 | 3.6 | 18.3 | 3.1 |

**Appendix N** Summary of values for selected seed constituent traits in desi chickpea (Continued).

| Genotype No. | TSW (g) | | Total starch (%) | | Amylose (%) | | Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 406 | 196.5 | 64.6 | 37.7 | 2.4 | 33.2 | 4.0 | 18.8 | 2.1 |
| 407 | 236.4 | 70.6 | 42.7 | 2.9 | 31.7 | 3.2 | 16.6 | 1.6 |
| 408 | 98.8 | 40.7 | 37.5 | 1.9 | 32.1 | 3.0 | 22.4 | 2.0 |
| 409 | 120.3 | 50.0 | 38.8 | 2.4 | 32.7 | 3.7 | 22.2 | 0.8 |
| 410 | 177.9 | 54.0 | 42.8 | 3.0 | 32.6 | 3.2 | 16.8 | 3.8 |
| 411 | 180.6 | 43.4 | 42.2 | 3.6 | 34.3 | 3.6 | 19.7 | 2.1 |
| 412 | 189.2 | 59.4 | 41.6 | 9.0 | 33.3 | 3.4 | 17.6 | 0.6 |
| 413 | 162.3 | 49.9 | 40.2 | 1.1 | 33.8 | 4.3 | 16.3 | 1.0 |
| 414 | 116.7 | 36.6 | 39.3 | 2.7 | 32.4 | 4.0 | 21.4 | 1.7 |
| 415 | 145.0 | 38.6 | 41.9 | 2.1 | 33.4 | 3.8 | 18.9 | 1.3 |
| 416 | 239.5 | 59.7 | 38.3 | 9.6 | 32.5 | 2.3 | 17.3 | 1.1 |
| 417 | 183.3 | 68.2 | 41.8 | 1.0 | 33.4 | 3.3 | 18.6 | 1.2 |
| 418 | 212.8 | 37.0 | 44.6 | 0.5 | 33.2 | 2.6 | 18.4 | 1.3 |
| 419 | 202.7 | 54.5 | 40.3 | 3.1 | 33.3 | 3.5 | 17.9 | 1.5 |
| 420 | 169.4 | 47.1 | 38.6 | 6.5 | 34.0 | 3.2 | 18.4 | 3.6 |
| 421 | 111.3 | 52.5 | 37.6 | 5.2 | 32.7 | 3.4 | 20.8 | 2.0 |
| 422 | 154.6 | 57.6 | 39.3 | 5.7 | 34.7 | 3.3 | 17.8 | 1.3 |
| 423 | 116.1 | 41.3 | 38.0 | 6.4 | 34.1 | 2.7 | 22.3 | 1.2 |
| 424 | 173.1 | 52.1 | 39.1 | 4.0 | 33.3 | 4.2 | 17.6 | 1.5 |
| 425 | 99.1 | 34.9 | 32.5 | 7.6 | 32.0 | 3.8 | 22.3 | 0.5 |
| 426 | 105.9 | 35.1 | 36.5 | 6.1 | 33.7 | 4.3 | 21.4 | 2.2 |
| 427 | 174.0 | 36.8 | 34.3 | 6.9 | 35.2 | 5.0 | 16.5 | 2.4 |
| 428 | 223.9 | 66.4 | 46.6 | 2.9 | 34.2 | 4.8 | 16.6 | 2.3 |
| 429 | 172.9 | 68.2 | 39.8 | 7.4 | 33.0 | 4.7 | 16.9 | 1.1 |
| 430 | 184.1 | 53.3 | 38.6 | 2.7 | 35.1 | 4.7 | 17.4 | 1.6 |
| 431 | 247.2 | 107.9 | 44.3 | 2.2 | 33.4 | 4.4 | 15.4 | 1.4 |
| 432 | 251.6 | 59.5 | 44.9 | 3.0 | 33.9 | 4.7 | 16.4 | 1.7 |
| 433 | 104.4 | 41.7 | 38.4 | 6.1 | 32.8 | 4.9 | 21.1 | 1.6 |
| 434 | 183.4 | 54.2 | 43.4 | 5.0 | 35.2 | 4.4 | 17.4 | 1.6 |
| 435 | 182.7 | 62.8 | 35.8 | 11.4 | 33.9 | 3.6 | 18.7 | 2.3 |
| 436 | 97.8 | 28.9 | 41.3 | 4.0 | 32.5 | 2.6 | 19.4 | 2.3 |
| 437 | 171.5 | 57.9 | 35.0 | 7.8 | 35.9 | 4.3 | 17.2 | 1.0 |
| 438 | 114.2 | 44.4 | 43.2 | 2.7 | 33.1 | 4.5 | 19.9 | 2.0 |
| 439 | 190.4 | 46.5 | 43.3 | 3.3 | 34.1 | 3.7 | 16.9 | 0.9 |
| 440 | 105.1 | 47.3 | 36.5 | 4.4 | 33.9 | 3.4 | 21.8 | 1.6 |
| 441 | 151.1 | 37.0 | 38.1 | 3.4 | 32.9 | 3.4 | 17.6 | 1.3 |
| 442 | 197.8 | 48.2 | 43.3 | 5.6 | 33.0 | 4.5 | 16.0 | 2.1 |
| 443 | 102.3 | 35.8 | 41.3 | 6.9 | 32.5 | 3.8 | 19.6 | 1.9 |
| 444 | 89.8 | 35.8 | 33.9 | 4.2 | 32.2 | 3.5 | 19.9 | 1.5 |
| 445 | 88.2 | 46.5 | 36.7 | 2.0 | 33.5 | 7.0 | 21.2 | 2.8 |
| 446 | 226.3 | 50.7 | 42.7 | 1.4 | 34.5 | 5.2 | 17.7 | 2.9 |
| 447 | 162.3 | 38.5 | 42.2 | 2.6 | 34.2 | 4.1 | 18.1 | 3.1 |
| 448 | 160.7 | 63.2 | 40.9 | 3.2 | 34.8 | 4.5 | 18.5 | 1.0 |
| 449 | 162.5 | 61.3 | 37.5 | 6.5 | 34.5 | 5.4 | 16.8 | 1.5 |
| 450 | 157.3 | 41.4 | 36.4 | 6.1 | 34.4 | 5.9 | 18.1 | 1.9 |

**Appendix N** Summary of values for selected seed constituent traits in desi chickpea (Continued).

| Genotype No. | TSW (g) | | Total starch (%) | | Amylose (%) | | Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 451 | 187.0 | 45.6 | 45.4 | 4.7 | 35.7 | 5.8 | 15.4 | 2.5 |
| 452 | 227.6 | 70.3 | 43.8 | 5.4 | 34.0 | 5.0 | 16.5 | 1.9 |
| 453 | 173.2 | 38.0 | 42.4 | 5.9 | 35.2 | 4.0 | 17.2 | 2.0 |
| 454 | 213.4 | 66.7 | 40.4 | 10.1 | 34.0 | 4.5 | 17.9 | 1.5 |
| 455 | 98.0 | 49.6 | 33.6 | 11.5 | 33.8 | 4.7 | 22.2 | 0.8 |
| 456 | 179.0 | 67.0 | 41.1 | 5.1 | 33.9 | 4.1 | 19.0 | 2.6 |
| 457 | 198.4 | 80.3 | 39.8 | 12.6 | 33.3 | 3.2 | 17.0 | 1.8 |
| 458 | 102.9 | 30.3 | 38.0 | 6.1 | 33.1 | 4.3 | 20.8 | 2.6 |
| 459 | 103.1 | 60.6 | 34.6 | 8.5 | 35.7 | 2.5 | 22.7 | 1.1 |
| 460 | 91.4 | 29.6 | 36.0 | 4.0 | 33.3 | 3.5 | 20.1 | 2.3 |
| 461 | 183.1 | 58.8 | 41.9 | 3.0 | 33.6 | 3.6 | 16.8 | 1.9 |
| 462 | 118.3 | 39.8 | 41.5 | 9.3 | 33.5 | 5.0 | 20.4 | 1.2 |
| 463 | 182.4 | 64.6 | 44.5 | 4.6 | 33.3 | 3.8 | 16.6 | 2.2 |
| 464 | 206.1 | 65.1 | 38.3 | 5.8 | 33.7 | 3.4 | 16.5 | 2.5 |
| 465 | 228.7 | 47.9 | 42.5 | 5.9 | 32.7 | 3.7 | 16.3 | 2.6 |
| 466 | 241.1 | 69.9 | 41.8 | 8.0 | 33.0 | 3.0 | 18.1 | 1.5 |
| 467 | 100.5 | 20.4 | 39.8 | 2.8 | 33.5 | 2.7 | 20.2 | 2.3 |
| 468 | 90.3 | 39.8 | 33.6 | 3.4 | 34.5 | 1.4 | 21.4 | 2.3 |
| 469 | 110.8 | 51.8 | 40.1 | 3.4 | 34.3 | 4.1 | 21.9 | 1.6 |
| 470 | 199.9 | 56.7 | 41.8 | 3.0 | 33.4 | 3.1 | 16.9 | 1.3 |
| 471 | 189.6 | 45.6 | 45.6 | 2.2 | 33.8 | 2.1 | 16.7 | 3.8 |
| 472 | 224.6 | 40.8 | 45.0 | 3.3 | 32.7 | 2.9 | 16.3 | 2.5 |
| 473 | 194.0 | 65.9 | 42.0 | 7.9 | 33.7 | 3.6 | 16.9 | 1.8 |
| 474 | 165.9 | 27.1 | 38.3 | 0.9 | 34.2 | 3.5 | 16.5 | 1.3 |
| 475 | 161.4 | 56.6 | 40.8 | 1.8 | 34.4 | 2.5 | 17.7 | 1.8 |
| 476 | 116.9 | 42.6 | 40.4 | 3.0 | 33.5 | 3.8 | 21.6 | 1.9 |
| 477 | 140.9 | 40.6 | 39.7 | 1.7 | 33.3 | 3.6 | 23.0 | 1.3 |
| 478 | 185.3 | 51.5 | 37.8 | 1.1 | 35.0 | 3.1 | 19.4 | 2.1 |
| 479 | 89.6 | 34.3 | 30.8 | 8.9 | 34.8 | 3.7 | 22.1 | 2.3 |
| 480 | 175.7 | 53.9 | 39.0 | 3.3 | 34.9 | 3.4 | 18.9 | 3.1 |
| 481 | 123.8 | 30.1 | 40.4 | 2.4 | 33.8 | 3.2 | 19.7 | 2.9 |
| 482 | 240.7 | 58.3 | 46.3 | 4.5 | 34.2 | 3.2 | 17.0 | 2.8 |
| 483 | 160.1 | 44.4 | 41.0 | 3.0 | 34.7 | 3.1 | 17.9 | 1.3 |
| 484 | 159.9 | 56.5 | 36.6 | 5.6 | 34.5 | 4.2 | 18.8 | 1.2 |
| 485 | 197.1 | 46.6 | 38.9 | 4.1 | 34.8 | 4.5 | 18.4 | 2.8 |
| 486 | 127.1 | 36.1 | 41.9 | 3.3 | 34.9 | 4.3 | 19.3 | 2.2 |
| 487 | 191.5 | 44.6 | 41.8 | 2.5 | 34.6 | 4.7 | 18.2 | 2.4 |
| 488 | 94.1 | 44.7 | 32.2 | 8.5 | 36.2 | 2.3 | 24.4 | 1.2 |
| 489 | 151.4 | 44.6 | 38.0 | 2.9 | 34.0 | 4.8 | 17.8 | 2.3 |
| 490 | 131.0 | 32.6 | 40.0 | 3.8 | 35.2 | 3.4 | 20.7 | 1.7 |
| 491 | 162.2 | 66.7 | 39.4 | 4.6 | 34.3 | 4.0 | 17.9 | 1.7 |
| 492 | 135.3 | 44.7 | 39.1 | 1.7 | 34.4 | 4.3 | 19.2 | 2.7 |
| 493 | 104.2 | 41.8 | 33.5 | 3.5 | 35.7 | 3.7 | 23.0 | 1.0 |
| 494 | 184.9 | 45.1 | 36.4 | 4.5 | 34.8 | 4.7 | 18.9 | 2.2 |
| 495 | 105.4 | 50.0 | 33.1 | 8.2 | 36.6 | 4.9 | 22.3 | 1.0 |

**Appendix N** Summary of values for selected seed constituent traits in desi chickpea (Continued).

| Genotype No. | TSW (g) | | Total starch (%) | | Amylose (%) | | Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 496 | 173.0 | 60.8 | 39.9 | 3.4 | 34.1 | 4.5 | 18.8 | 2.3 |
| 497 | 199.0 | 66.9 | 44.7 | 0.9 | 33.4 | 4.4 | 16.8 | 0.6 |
| 498 | 111.9 | 31.2 | 33.6 | 4.3 | 34.3 | 3.1 | 22.5 | 3.5 |
| 499 | 225.7 | 83.1 | 38.5 | 10.7 | 33.7 | 3.3 | 17.3 | 1.6 |
| 500 | 221.5 | 60.8 | 43.6 | 0.8 | 34.3 | 3.4 | 16.7 | 2.6 |
| 501 | 195.2 | 41.1 | 39.5 | 4.4 | 34.3 | 3.6 | 19.0 | 3.8 |
| 502 | 204.8 | 31.5 | 39.2 | 3.8 | 34.9 | 3.3 | 18.9 | 3.7 |
| 503 | 168.6 | 49.6 | 34.3 | 6.1 | 34.6 | 4.1 | 18.8 | 1.9 |
| 504 | 186.8 | 48.5 | 40.3 | 6.6 | 34.5 | 3.7 | 18.5 | 2.7 |
| 505 | 202.8 | 47.9 | 44.5 | 5.1 | 33.2 | 5.2 | 18.3 | 2.3 |
| 506 | 175.4 | 35.1 | 42.1 | 1.1 | 33.6 | 4.9 | 17.4 | 2.5 |
| 507 | 209.3 | 77.1 | 43.9 | 4.3 | 32.8 | 5.3 | 16.1 | 1.9 |
| 508 | 184.0 | 39.0 | 37.3 | 5.1 | 35.2 | 6.1 | 18.0 | 2.7 |
| 509 | 97.8 | 54.5 | 28.0 | 10.1 | 36.9 | 3.4 | 23.9 | 2.3 |
| 510 | 120.4 | 54.6 | 38.5 | 6.6 | 34.5 | 5.1 | 24.1 | 1.7 |
| 511 | 107.8 | 39.0 | 33.0 | 1.4 | 34.7 | 4.7 | 20.8 | 1.5 |
| 512 | 185.3 | 51.9 | 44.0 | 1.5 | 33.1 | 5.1 | 15.4 | 2.0 |
| 513 | 112.0 | 38.2 | 36.2 | 6.3 | 34.2 | 3.5 | 22.7 | 2.5 |
| 514 | 204.8 | 41.9 | 38.0 | 1.7 | 34.7 | 4.7 | 18.9 | 3.0 |
| 515 | 229.8 | 71.7 | 42.7 | 3.3 | 33.4 | 4.8 | 17.0 | 3.1 |
| 516 | 105.2 | 49.0 | 34.8 | 8.5 | 34.3 | 5.3 | 23.5 | 1.0 |
| 517 | 130.6 | 37.5 | 40.2 | 3.8 | 33.3 | 5.4 | 21.5 | 2.8 |
| 518 | 197.5 | 66.9 | 42.7 | 2.6 | 32.4 | 5.3 | 16.8 | 1.1 |
| 519 | 118.0 | 55.2 | 38.1 | 4.2 | 34.3 | 4.4 | 24.0 | 2.2 |

**Appendix O** Summary of values for selected seed constituent traits in kabuli chickpea.

| Genotype No. | TSW (g) | | Total starch (%) | | Amylose (%) | | Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 1 | 246.3 | 28.3 | 41.5 | 4.4 | 30.1 | 1.7 | 21.1 | 2.0 |
| 2 | 344.1 | 36.9 | 47.2 | 4.7 | 30.7 | 1.8 | 19.9 | 4.8 |
| 3 | 272.5 | 13.1 | 44.4 | 6.5 | 30.7 | 3.2 | 24.0 | 2.3 |
| 4 | 200.2 | 1.7 | 38.6 | 2.3 | 33.3 | 3.7 | 20.2 | 0.6 |
| 5 | 151.2 | 34.3 | 36.2 | 2.0 | 30.8 | 1.5 | 21.7 | 3.3 |
| 6 | 198.2 | 26.1 | 41.7 | 4.0 | 33.6 | 2.9 | 23.1 | 3.5 |
| 7 | 116.7 | 18.2 | 37.6 | 2.6 | 34.0 | 2.8 | 21.5 | 4.5 |
| 8 | 185.2 | 18.1 | 40.8 | 3.6 | 33.6 | 3.5 | 24.3 | 6.3 |
| 9 | 172.3 | 31.2 | 40.0 | 3.4 | 34.0 | 2.5 | 24.8 | 4.1 |
| 10 | 276.7 | 18.8 | 42.9 | 4.5 | 31.2 | 2.7 | 20.7 | 4.0 |
| 11 | 291.5 | 28.5 | 42.2 | 3.1 | 33.7 | 2.5 | 23.0 | 3.7 |
| 12 | 279.7 | 33.0 | 42.9 | 3.1 | 31.3 | 2.1 | 21.5 | 4.5 |
| 13 | 146.8 | 26.9 | 37.2 | 0.8 | 31.8 | 1.6 | 19.5 | 3.0 |
| 14 | 227.8 | 118.2 | 45.2 | 0.6 | 33.5 | 3.3 | 18.6 | 3.4 |
| 15 | 264.4 | 81.1 | 43.5 | 1.2 | 34.0 | 1.7 | 17.8 | 0.9 |
| 16 | 273.7 | 61.4 | 45.7 | 3.9 | 35.0 | 1.7 | 20.0 | 3.1 |
| 17 | 276.0 | 29.6 | 45.4 | 3.2 | 33.7 | 3.6 | 20.0 | 4.1 |
| 18 | 258.9 | 23.5 | 42.8 | 2.6 | 33.0 | 4.2 | 21.5 | 4.7 |
| 19 | 191.1 | 55.6 | 43.6 | 0.2 | 32.6 | 0.3 | 17.0 | 3.2 |
| 20 | 196.8 | 23.7 | 42.6 | 0.6 | 34.6 | 2.4 | 21.1 | 3.3 |
| 21 | 300.2 | 41.5 | 42.9 | 3.7 | 32.9 | 2.0 | 20.8 | 5.5 |
| 22 | 294.7 | 34.0 | 42.5 | 2.0 | 31.4 | 1.5 | 20.5 | 5.2 |
| 23 | 128.5 | 36.0 | 43.0 | 3.4 | 29.2 | 1.4 | 22.4 | 3.6 |
| 24 | 335.5 | 39.8 | 45.1 | 2.0 | 33.4 | 2.5 | 20.7 | 5.0 |
| 25 | 131.5 | 38.5 | 41.8 | 1.5 | 29.9 | 0.9 | 22.6 | 2.9 |
| 26 | 250.6 | 41.4 | 40.8 | 2.0 | 32.2 | 3.2 | 22.8 | 5.2 |
| 27 | 142.1 | 22.7 | 37.0 | 5.1 | 32.3 | 0.1 | 22.7 | 3.3 |
| 28 | 326.2 | 27.7 | 43.5 | 3.9 | 32.3 | 1.8 | 20.6 | 4.3 |
| 29 | 243.7 | 64.7 | 44.2 | 1.1 | 30.6 | 0.8 | 18.2 | 1.4 |
| 30 | 312.6 | 43.6 | 43.4 | 3.7 | 32.5 | 0.9 | 20.8 | 3.0 |
| 31 | 179.4 | 85.1 | 40.1 | 1.6 | 32.2 | 2.0 | 21.8 | 3.9 |
| 32 | 211.1 | 92.1 | 44.7 | 5.4 | 32.1 | 2.9 | 20.4 | 4.1 |
| 33 | 115.7 | 24.3 | 37.6 | 1.6 | 31.1 | 2.6 | 23.2 | 3.2 |
| 34 | 308.0 | 35.5 | 43.0 | 1.3 | 32.6 | 3.7 | 19.7 | 4.4 |
| 35 | 257.0 | 42.6 | 46.3 | 6.5 | 32.4 | 2.8 | 21.5 | 4.1 |
| 36 | 238.6 | 49.4 | 42.7 | 2.2 | 33.0 | 4.6 | 21.9 | 5.3 |
| 37 | 394.9 | 30.2 | 42.4 | 1.9 | 31.8 | 3.2 | 20.4 | 4.4 |
| 38 | 348.0 | 37.4 | 43.1 | 1.3 | 31.2 | 2.7 | 21.1 | 3.7 |
| 39 | 296.2 | 71.0 | 45.9 | 0.5 | 32.9 | 1.2 | 18.8 | 1.1 |
| 40 | 209.2 | 33.3 | 41.7 | 3.5 | 32.6 | 1.4 | 22.7 | 4.0 |
| 41 | 278.9 | 48.1 | 49.0 | 1.3 | 32.1 | 0.7 | 19.8 | 3.0 |
| 42 | 310.9 | 40.5 | 46.5 | 2.1 | 32.8 | 1.4 | 20.5 | 3.9 |
| 43 | 322.3 | 46.1 | 44.8 | 0.7 | 32.4 | 0.9 | 21.0 | 3.1 |
| 44 | 344.4 | 46.3 | 45.1 | 1.1 | 32.3 | 0.8 | 19.8 | 3.5 |
| 45 | 537.4 | 37.8 | 46.1 | 4.1 | 31.4 | 1.4 | 20.7 | 0.3 |

**Appendix O** Summary of values for selected seed constituent traits in kabuli chickpea (Continued).

| Genotype No. | TSW (g) | | Total starch (%) | | Amylose (%) | | Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 46 | 314.2 | 66.8 | 41.6 | 1.5 | 31.5 | 0.2 | 17.8 | 2.0 |
| 47 | 330.2 | 27.5 | 45.9 | 1.0 | 31.9 | 0.6 | 21.6 | 5.1 |
| 48 | 335.8 | 61.4 | 47.0 | 1.5 | 32.1 | 0.5 | 19.8 | 3.0 |
| 49 | 352.5 | 35.4 | 45.4 | 1.5 | 31.8 | 1.6 | 20.6 | 2.4 |
| 50 | 306.3 | 38.4 | 43.7 | 4.8 | 32.3 | 0.4 | 21.6 | 3.6 |
| 51 | 301.9 | 33.4 | 44.9 | 1.0 | 32.1 | 1.3 | 22.2 | 2.7 |
| 52 | 370.4 | 31.8 | 46.9 | 2.2 | 31.5 | 0.7 | 20.8 | 3.3 |
| 53 | 412.9 | 19.4 | 46.4 | 0.5 | 31.4 | 0.5 | 20.8 | 3.9 |
| 54 | 329.6 | 46.9 | 41.1 | 3.4 | 33.4 | 2.1 | 21.9 | 2.0 |
| 55 | 219.3 | 37.3 | 51.6 | 8.3 | 35.6 | 2.2 | 23.9 | 3.4 |
| 56 | 182.7 | 24.4 | 48.7 | 2.1 | 34.2 | 2.5 | 23.8 | 2.7 |
| 57 | 235.7 | 10.6 | 47.0 | 5.8 | 34.6 | 3.3 | 22.8 | 4.1 |
| 58 | 171.2 | 26.2 | 45.0 | 1.0 | 35.0 | 3.0 | 26.0 | 2.1 |
| 59 | 265.2 | 43.8 | 52.0 | 5.7 | 33.7 | 3.6 | 22.9 | 1.2 |
| 60 | 130.7 | 22.4 | 46.7 | 5.9 | 34.2 | 4.0 | 24.1 | 2.8 |
| 61 | 264.4 | 33.3 | 53.5 | 5.6 | 34.8 | 3.6 | 22.3 | 2.1 |
| 62 | 264.3 | 52.2 | 51.8 | 5.0 | 33.4 | 3.4 | 25.1 | 1.1 |
| 63 | 316.8 | 30.3 | 51.2 | 4.8 | 34.3 | 3.9 | 23.2 | 2.2 |
| 64 | 304.0 | 37.6 | 56.4 | 6.4 | 32.6 | 4.4 | 20.1 | 1.8 |
| 65 | 222.2 | 35.8 | 49.6 | 2.9 | 32.0 | 2.2 | 24.5 | 0.2 |
| 66 | 311.8 | 10.7 | 53.0 | 4.0 | 33.0 | 3.0 | 22.1 | 0.5 |
| 67 | 252.8 | 30.4 | 48.3 | 2.1 | 33.4 | 1.9 | 23.1 | 0.8 |
| 68 | 302.8 | 55.5 | 53.3 | 7.5 | 33.1 | 3.3 | 21.5 | 3.1 |
| 69 | 237.9 | 15.3 | 47.1 | 4.2 | 34.2 | 4.8 | 21.8 | 1.6 |
| 70 | 298.7 | 23.2 | 50.8 | 6.4 | 32.3 | 1.5 | 22.9 | 3.4 |
| 71 | 331.4 | 38.1 | 48.1 | 2.4 | 33.1 | 2.4 | 23.6 | 1.6 |
| 72 | 186.8 | 20.9 | 52.0 | 7.3 | 32.8 | 2.4 | 22.6 | 2.8 |
| 73 | 176.3 | 33.9 | 53.0 | 2.4 | 31.6 | 2.5 | 24.4 | 1.1 |
| 74 | 208.6 | 21.4 | 47.6 | 1.3 | 33.2 | 2.4 | 23.0 | 2.0 |
| 75 | 167.7 | 17.3 | 46.7 | 1.8 | 31.7 | 2.8 | 24.2 | 2.9 |
| 76 | 186.3 | 13.7 | 51.1 | 4.9 | 32.3 | 3.3 | 22.7 | 3.6 |
| 77 | 181.5 | 20.1 | 49.1 | 2.7 | 31.8 | 1.7 | 22.4 | 2.0 |
| 78 | 109.7 | 4.4 | 45.5 | 3.6 | 31.6 | 1.2 | 23.8 | 2.2 |
| 79 | 319.3 | 9.5 | 46.5 | 2.5 | 32.9 | 2.1 | 21.2 | 2.6 |
| 80 | 306.8 | 37.8 | 51.3 | 4.3 | 32.2 | 3.3 | 23.5 | 0.9 |
| 81 | 395.5 | 138.0 | 50.4 | 1.6 | 31.1 | 4.2 | 23.4 | 2.4 |
| 82 | 287.5 | 9.8 | 51.4 | 1.4 | 33.9 | 2.4 | 21.0 | 3.3 |
| 83 | 141.2 | 21.3 | 39.0 | 4.3 | 33.9 | 3.8 | 22.5 | 1.5 |
| 84 | 258.7 | 10.3 | 49.5 | 1.4 | 33.7 | 4.5 | 20.6 | 2.3 |
| 85 | 258.8 | 53.0 | 47.7 | 4.9 | 33.3 | 4.6 | 20.6 | 3.9 |
| 86 | 256.6 | 42.1 | 48.2 | 4.3 | 32.5 | 3.0 | 22.1 | 2.4 |
| 87 | 298.6 | 15.3 | 52.8 | 6.8 | 33.6 | 3.7 | 20.6 | 2.9 |
| 88 | 250.5 | 9.4 | 46.9 | 5.0 | 34.2 | 3.6 | 21.1 | 2.5 |
| 89 | 184.7 | 19.2 | 43.4 | 5.9 | 33.4 | 2.6 | 22.7 | 1.1 |
| 90 | 171.0 | 2.1 | 49.6 | 5.3 | 33.6 | 3.2 | 25.1 | 2.2 |

**Appendix O** Summary of values for selected seed constituent traits in kabuli chickpea (Continued).

| Genotype No. | TSW (g) | | Total starch (%) | | Amylose (%) | | Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 91 | 212.1 | 27.4 | 47.7 | 3.8 | 33.8 | 3.0 | 22.8 | 1.8 |
| 92 | 171.6 | 14.9 | 46.6 | 1.9 | 32.6 | 2.7 | 20.3 | 4.1 |
| 93 | 253.3 | 37.3 | 47.7 | 3.2 | 34.1 | 4.2 | 21.7 | 3.5 |
| 94 | 158.1 | 10.5 | 48.9 | 0.9 | 34.5 | 2.7 | 23.5 | 1.9 |
| 95 | 228.9 | 45.8 | 45.8 | 2.6 | 33.4 | 2.6 | 24.6 | 3.1 |
| 96 | 323.1 | 27.5 | 48.6 | 1.3 | 33.2 | 3.5 | 21.4 | 1.9 |
| 97 | 262.2 | 18.6 | 51.5 | 0.7 | 32.8 | 4.3 | 21.2 | 1.7 |
| 98 | 337.9 | 46.6 | 49.9 | 3.4 | 34.2 | 3.2 | 20.1 | 2.5 |
| 99 | 312.4 | 55.2 | 45.1 | 5.9 | 34.2 | 3.2 | 21.6 | 2.1 |
| 100 | 282.6 | 16.1 | 46.3 | 1.7 | 34.5 | 3.1 | 21.5 | 3.7 |
| 101 | 314.8 | 45.4 | 41.8 | 5.9 | 33.4 | 3.7 | 23.0 | 0.3 |
| 102 | 347.7 | 35.6 | 46.2 | 4.7 | 35.7 | 2.1 | 21.4 | 2.6 |

**Appendix P** Summary of values for selected seed constituent traits in pea-shaped chickpea.

| Genotype No. | TSW (g) | | Total starch (%) | | Amylose (%) | | Protein (%) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 1 | 229.5 | 40.7 | 38.2 | 4.6 | 36.1 | 3.7 | 21.4 | 1.6 |
| 2 | 173.8 | 13.5 | 41.1 | 3.1 | 32.7 | 1.8 | 20.5 | 2.4 |
| 3 | 251.8 | 16.3 | 41.1 | 5.7 | 35.5 | 3.3 | 22.6 | 2.6 |
| 4 | 173.5 | 6.7 | 41.2 | 3.9 | 34.3 | 1.6 | 22.6 | 3.4 |
| 5 | 174.7 | 14.4 | 41.6 | 4.0 | 34.2 | 0.7 | 22.7 | 3.0 |
| 6 | 168.1 | 3.6 | 43.1 | 6.2 | 35.4 | 2.2 | 22.7 | 1.5 |
| 7 | 226.2 | 31.9 | 43.0 | 3.2 | 34.8 | 2.3 | 22.0 | 2.3 |
| 8 | 168.7 | 28.3 | 44.7 | 4.6 | 37.5 | 4.3 | 22.2 | 2.2 |